Severe feather-pecking in free-range ISA Brown laying hens

by

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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2016

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ACKNOWLEDGEMENTS

I have had the extreme good fortune of being surrounded by supportive and encouraging people, who have nurtured my personal and professional development throughout my PhD. I am indebted to my family, friends, colleagues, and mentors.

I am grateful to all my supervisors. Greg, my primary supervisor, has given me countless opportunities, endless encouragement and support, and without whom, this thesis would not have been possible. From the beginning of my candidature, Greg has provided me with opportunities and always encouraged me to make my own choices and commit to my decisions, fostering independent research. Stuart enthusiastically accepted to come on board as an associate supervisor despite an initial disparity between our fields of research. Stuart has also been exceptionally supportive, encouraging, generous with his time, and gracious. Paul has given insightful and inspirational contributions to all chapters of this thesis. Every chapter is more robust, eloquent and refined due to Paul’s input. Mini has given me the opportunity to become involved in research that I would not have otherwise been able to do, and has always been welcoming and supportive.

I am very appreciative of Peter Thomson for his generous help, patience, and exceptional knowledge on statistical methods. This thesis would not have been possible without Peter’s advice and guidance. Thank you to Mary, originally my research assistant and co-conspirator, now my close friend. Mary was instrumental in many of the practical aspects of this thesis. Thank you to all the staff at the Poultry CRC for their ongoing support. The CRC’s investment in their students results in furthered professional development, and a more well-rounded research

experience. I am very appreciative of their efforts. Similarly, the AECL have been very supportive. I am grateful for the opportunities that I have been given throughout my candidature.

Thank you to Tugrul, who I didn’t see much during my candidature, but who had a big impact on me by his generosity, friendliness and eagerness to help. Thank you to Carlos, who I met at the beginning of my candidature, and who inspired me with his passion for animal behaviour research and uplifting attitude. Thank you to Peter Groves, who always made sure to check that I was doing okay, and to offer help. Thank you to Jeff Downing, my supervisor during my honours project, who supported my application to start my postgraduate work, and patiently donated his lab and freezer space.

In the second year of my candidature I visited some researchers in Europe was taken aback by the generosity that I experienced during my trip. I was lucky to be able to visit and learn from researchers whom I respect. In order of my trip, thank you to Claire Weeks, Ruth Llayton, Ashley Bright, Jasper Heerkens, Frank Tuyttens, Bas Rodenburg, Elske de Haas, Werner Bessei and Stefanie Bogelein. It was a pleasure to spend time with all of them, including others who I have not listed here. I learnt a lot from all of them and I am thankful for the time they gave to spend with me. Thank you also to Inma Estevez, Ruth Newberry, Antoni Dalmau and Nicolau Casal for similarly taking the time to host me on my visit to Spain last year.

Thank you to my family. My brother Dylan reliably keeps me down to earth, and is always quick to remind me that my studies are nothing more than watching painted birds run around a shed in a blur of colour which is still too much for my simplicity. I think Dad has enjoyed my topic of research as it has allowed him to come up with countless chicken puns. Dad has been extremely generous in offering to help. Thank you to Mum, who is always supportive and understanding, and to Colin for taking an active and genuine interest in my work, myself, and for Sunday night family dinners. Thank you to my little sister Nina, who had to make an adjustment to seeing me less due to my long work hours and distance from home, and who has visited me out in Camden a number of times. I am lucky to have a supportive family who all take an active interest in my work. Thank you to my partner and fellow PhD candidate, Dean, for his unwavering support. He has been a true example of patience, calmness, and resilience, whose strong nature I admire.

Thank you to the chickens, those in my experiments as well as in the industry, for whom this research was conducted. Chickens currently endure serious compromises to their welfare in all types of housing systems. The welfare of production animals is an exceptionally important issue and is one which is prone to be forgotten as it is not evident in our everyday lives.

DECLARATION OF AUTHORSHIP

The studies presented in this thesis are the result of original investigations completed by the author while a post-graduate student in the Faculty of Veterinary Science, University of Sydney. The studies have not been previously submitted for any other degree or diploma. I further certify that any assistance with the preparation of this thesis and all sources used have been appropriately acknowledged.

Kate Hartcher

September 2015

ACKNOWLEDGMENT OF CONTRIBUTION TO THE RESEARCH WORK AND AUTHORSHIP

This thesis includes five original research papers and one review, which have been published in, or submitted to, peer-reviewed journals. The core theme of the thesis is severe feather-pecking in free-range laying hens and some causal factors.

The ideas, development, and writing of all chapters in this thesis were the principal responsibility of the candidate, Miss Kate Hartcher, working within the Faculty of Veterinary Science under the supervision of Dr. Greg Cronin (primary supervisor), Prof. Paul Hemsworth (associate supervisor), Dr. Stuart Wilkinson (associate supervisor), and Dr. Mini Singh (associate supervisor).

The inclusion of co-authors reflects that the work came from active collaboration between researchers, and acknowledges input into team-based research.

CONFIRMATION OF CO-AUTHORSHIP

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Kate M. Hartcher, Stuart J. Wilkinson, Paul H. Hemsworth, Greg M. Cronin

Severe feather-pecking in non-cage laying hens and some associated and predisposing factors: a review.

Authors' contributions

KMH wrote the manuscript, SJW, PHH and GMC were involved in editing, collaborating, and preparing the manuscript for publication.

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The effects of environmental enrichment and beak-trimming during the rearing period on subsequent feather damage due to feather-pecking in laying hens.

Authors' contributions

In order of author listing: KMH conducted all testing and observing, data collection, experimental and methodological planning, data consolidation, data analyses, writing of the manuscript, and responses to reviewers' comments. MKTNT assisted with practical work including data collection and experimental planning. GMC and SJW assisted with some practical work, and feedback on preparation of the manuscript. GMC and PHH conceived the initial experimental design, and provided input on preparation of the manuscript. PCT assisted by consultation for data analyses and providing feedback on the manuscript.

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Plumage damage in free-range laying hens: Behavioural characteristics in the rearing period and the effects of environmental enrichment and beak-trimming.

Authors' contributions

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Authors’ contributions

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Chapter 7

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Relationships between range access as monitored by radio frequency identification (RFID) technology, fearfulness, and plumage damage in free-range laying hens.

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PREFACE

This thesis has been written in publication style. Chapters 2 to 7 are therefore stand-alone manuscripts, each with its own abstract, introduction, materials and methods (where applicable), results, discussion, and conclusion. Chapters 2, 3, 4, 6, and 7 have been published in peer-reviewed journals, and have been re-formatted for this thesis. K. M. Hartcher is the first author on all chapters. This information is indicated accordingly on the cover page for each chapter. Assistance given by others is indicated in the Acknowledgement of Contribution statement, and in the Acknowledgments of each chapter.

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

Kate Hartcher

September 2015

LIST OF ABBREVIATIONS

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

FP	Feather-pecking
SFP	Severe feather-pecking
GFP	Gentle feather-pecking
BT	Beak-trimming
EE	Environmental enrichment
OFT	Open field test
TI	Tonic immobility
h	hour(s)
s	second(s)
min	minute(s)

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CHAPTER 7: Relationships between range access as monitored by radio frequency identification (RFID) technology, fearfulness, and plumage damage in free-range laying hens.

JOURNAL PUBLICATIONS RELATING TO THESIS

Hartcher, K. M., K. T. N. Tran, S. J. Wilkinson, P. H. Hemsworth, P. C. Thomson, and G. M. Cronin. 2015. The effects of environmental enrichment and beak-trimming during the rearing period on subsequent feather damage due to feather-pecking in laying hens. Poultry Science. 94:852-859.

Hartcher, K. M., M. K. T. N. Tran, S. J. Wilkinson, P. H. Hemsworth, P. C. Thomson, and G. M. Cronin. 2015. Plumage damage in free-range laying hens: Behavioural characteristics in the rearing period and the effects of environmental enrichment and beak-trimming. Applied Animal Behaviour Science. 164:64-72.

Hartcher, K. M., K. A. Hickey, P. H. Hemsworth, G. M. Cronin, S. J. Wilkinson, and M. Singh. 2015. Relationships between range access as monitored by radio frequency identification (RFID) technology, fearfulness, and plumage damage in free-range laying hens. animal. 23:1-7.

Hartcher, K. M., P. H. Hemsworth, S. J. Wilkinson, P. C. Thomson, and G. M. Cronin. 2015. The association between plumage damage and feather-eating in free-range laying hens. animal. doi:10.1017/S1751731115002608.

Hartcher, K. M., S. J. Wilkinson, P. H. Hemsworth, and G. M. Cronin. 2016. Severe feather-pecking in non-cage laying hens and some associated and predisposing factors: A review. World's Poultry Science Journal. 72:103-114.

CONFERENCE PROCEEDINGS AND ABSTRACTS RELATING TO THESIS

Peer-reviewed Conference Abstracts

Hartcher, K., Tran, M., Wilkinson, S., Hemsworth, P., Cronin, G. (2012). Effects of rearing conditions on the development of feather-pecking behaviour in free-range laying hens. Regional International Society for Applied Ethology Meeting: Our Animals, Our Issues, Parkville, Victoria, Australia.

Hartcher, K., Tran, M., Wilkinson, S., Hemsworth, P., Cronin, G. (2013). Feather-pecking in free-range laying hens: effects of environmental enrichment and beak-trimming in rearing. World's Poultry Science Association 9th European Symposium on Poultry Welfare, Uppsala, Sweden.

Hartcher, K., Tran, K., Wilkinson, S., Hemsworth, P., Thomson, P., Cronin, G. (2013). Feather-pecking in free-range laying hens. Australasian Veterinary Poultry Association, Adelaide, Australia.

Hartcher, K., Tran, K., Wilkinson, S., Hemsworth, P., Thomson, P., Cronin, G. (2013). Effect of rearing conditions on the development of feather-pecking behaviours in free-range laying hens. 24th Annual Australian Poultry Science Symposium, Sydney, Australia.

Cronin, G., Tran, K., **Hartcher, K**. (2013). The effects of beak-trimming and environmental enrichment during rearing on egg laying by free range hens during early lay. 24th Annual

Australian Poultry Science Symposium, Sydney, Australia: Poultry Research Foundation, University of Sydney, Australia.

Hartcher, K., Wilkinson, S., Hemsworth, P., Cronin, G. (2014). The association between feather-eating and severe feather-pecking in free-range laying hens. International Society for Applied Ethology 48th International Congress: Moving on, Vitoria-Gasteiz, Spain.

Hartcher, K., Wilkinson, S., Hemsworth, P., Cronin, G. (2014). The attractiveness of loose feathers to free-range laying hens exhibiting severe feather-pecking. 25th Annual Australian Poultry Science Symposium, Sydney, Australia: Poultry Research Foundation, University of Sydney, Australia.

Hartcher, K., Hickey, K., Hemsworth, P., Cronin, G. Singh, M. (2015). The association between plumage condition, fearfulness and range use as monitored by radio frequency identification, in free-range laying hens. International Society for Applied Ethology 49th International Congress: Ethology for Sustainable Society, Sapporo Hokkaido, Japan.

Hartcher, K., Hickey, K., Hemsworth, P., Cronin, G. Singh, M. (2016). Is range use related to fearfulness and plumage damage? 27th Annual Australian Poultry Science Symposium, Sydney, Australia: Poultry Research Foundation, University of Sydney, Australia.

Non Peer-reviewed Conference Abstracts

Hartcher, K., Wilkinson, S., Hemsworth, P., Cronin, G. (2012). Behaviours in free-range laying hens. Postgraduate conference, Sydney, Australia.

Hartcher, K., Welfare and production: Walking the balance beam (2014). Poultry Cooperative Research Centre Ideas Exchange: The Changing Landscape of Poultry Production, Gold Coast, Australia.

Hartcher, K., Wilkinson, S., Hemsworth, P., Cronin, G. (2014). The association between feather-eating and severe feather pecking in free-range laying hens. Postgraduate conference, Sydney, Australia.

Hartcher, K., Wilkinson, S., Hemsworth, P., Cronin, G. (2015). Severe feather-pecking in free-range laying hens housed in an experimental facility – some predisposing factors. 21st Australian Egg Corporation Limited Industry Forum, Adelaide, Australia.

AWARDS AND TRAVEL GRANTS RELATING TO THESIS

Poultry Cooperative Research Centre Postgraduate Scholarship (2012-15)

Australian Egg Corporation Limited Travel Grant (2013)

Poultry Cooperative Research Centre Travel Grant (2013)

Publication Prize, University of Sydney (2014)

Australian Egg Corporation Limited Travel Grant (2014)

Postgraduate Research Support Scheme, University of Sydney Travel Grant (2014)

TJ Robinson Travelling Fellowship, Research Development Committee, University of Sydney (2014)

Australian Egg Corporation Limited Travel Grant (2015)

ABSTRACT

Severe feather-pecking (SFP) is a multifactorial behaviour whereby birds peck at and pull out the feathers of conspecifics. It can result in extensive feather loss, wounds, and death in affected birds. Many causal factors have been identified in contributing to the expression of SFP, yet the underlying motivations for the behaviour are not fully understood. Despite over five decades of research, SFP persists as a highly deleterious and prevalent problem in the egg industry worldwide, with no effective method of control. SFP can occur at extremely high rates, with studies reporting between half and three-quarters of flocks surveyed as affected. SFP therefore represents a significant risk to hen welfare.

In the last half century, increased intensification of animal production has taken place. Concurrently over recent years, there has been an increase in non-cage housing systems for laying hens. Since SFP is thought to be socially transmitted throughout a flock, it has been found to be particularly difficult to control in non-cage systems. SFP is thought to be inversely correlated with use of the outdoor range area, although few studies have investigated this relationship and factors which affect range use. Research to investigate why SFP occurs, and how it may be managed, is critical for the future of the egg industry, as well as hen welfare.

Some research suggests that SFP is an abnormal behaviour, and may be used as an animal model for obsessive compulsive disorder in humans. Other studies hypothesise that it is triggered by the inhibition of ground-pecking, a foraging behaviour. Still others suggest that SFP has a nutritional basis, and is primarily performed with the intent to ingest feathers. Fearfulness is thought to play a role in the expression of SFP, but the causal relationship has not been determined, as well as whether the recipients or those performing SFP are more fearful. It is also not clear whether the gentle form of feather-pecking (GFP), which does not cause damage to the birds, acts as a precursor to SFP later in life. SFP has been widely controlled by beak-trimming (BT), whereby the tip of the beak is removed. BT does not address the cause of the behaviour, and is the subject of controversy and banned in a number of countries due to welfare reasons. Its effectiveness has also been questioned. There is a need for research to investigate the current need for BT, and potential alternatives.

Chapter 1 gives a general introduction to the topics covered in this thesis. Chapter 2 provides a consolidated review of the research on the motivational basis for SFP, how SFP is affected by the environment, and how feeding behaviour and feather-eating are implicated. It was concluded in chapter 2 that more research should be conducted to investigate types of environmental enrichment (EE) that may enhance foraging behaviours as a method to reduce SFP. In addition, research is required on individual bird variation, and the underlying behavioural characteristics related to the expression of SFP. Further, while feather-eating is thought to be important in the expression of SFP, more research is required to understand the specific role it plays, and why some birds may have a greater appetite for feathers.

Chapter 3 studies the effects of BT and EE during the rearing period, and plumage damage due to SFP later in life. Chapter 4 investigates some behavioural traits of pullets during the rearing period, how these are affected by the BT and EE treatments, and whether they may be predictive of SFP later in life. Sixteen pens of 50 ISA Brown laying hens were used (n = 800). The BT and EE treatments were applied in a 2 × 2 factorial arrangement. Half of the birds were BT at 1 d of age using an infra-red laser, and a follow-up light-trim was performed at 11 weeks of age with a hot blade. Half of the birds received EE which comprised pecking strings, whole oats in the litter, and greater litter depth. Four focal birds were selected at random per pen, at 11 days of age (n = 64). In chapter 3, focal birds were subject to *in situ* behaviour observations from 3 to 14 weeks of age. BT birds performed less ground-pecking ($P = 0.003$), less SFP ($P = 0.02$) and more GFP ($P = 0.02$) than their non-BT counterparts during the rearing period. These birds also exhibited less feather damage at 43 weeks of age ($P < 0.001$). EE caused birds to perform more ground-scratching ($P = 0.03$) and more dustbathing ($P = 0.01$) during the rearing period, but had no effect on plumage damage at 43 weeks of age. There was also no interaction effect of BT and EE on plumage damage. It was concluded that BT was an effective method to control SFP and plumage damage, and while pullet behaviour was modified by EE during rearing, it was not effective in reducing SFP.

In chapter 4, focal birds were subjected to well-validated behaviour tests; the open-field test (OFT) and the tonic immobility (TI) test, at 5 and 9 weeks of age, respectively. These tests were performed to estimate fearfulness, coping style, and social motivation. Non-BT birds vocalised more ($P = 0.02$) and at louder volumes ($P = 0.02$) in the OFT at 5 weeks of age, potentially indicating a higher need for social reinstatement. There were no differences between treatments in their levels of fearfulness, as assessed by the TI test ($P = 0.99$). There were no predictive relationships between test responses at 5 weeks of age, and plumage damage at 43 weeks of age when analysed using ordinal regression. It was concluded that while the behavioural tests were not related to plumage damage later in life, BT may cause a decreased need for social reinstatement at a young age.

Leading on from chapter 4, chapter 5 investigates some underlying behavioural traits of hens classified as feather-peckers, victims, or controls (n = 70). Focal birds were selected due to their involvement in SFP. Victims were selected due to extensive plumage damage, feather-peckers were selected due to the performance of SFP towards other birds, and controls were selected due to a lack of plumage damage and SFP. Control birds thereby appeared unaffected by SFP. The victims and feather-peckers were in pens in which SFP and plumage damage were evident, whereas the control birds were in pens in which little plumage damage was evident. Focal birds were subjected to an OFT and a TI test in the laying period, between 35 and 42 weeks of age, and observed in their home pens at 43 and 44 weeks of age. Birds in SFP pens performed ($P = 0.01$) and received ($P < 0.001$) more SFP, and also performed more GFP ($P = 0.007$) than non-SFP pens, but there was no relationship between SFP in a pen and ground-pecking ($P > 0.1$). In the OFT, feather-peckers made more vocalisations ($P < 0.001$) at higher volumes ($P < 0.001$) than ‘victims’ of SFP, and also made more escape attempts ($P = 0.03$). In the TI test, victims had longer latencies to right themselves than peckers ($P < 0.001$). It was concluded that the higher levels of vocalisations and escape attempts made by peckers in the OFT may have been due to greater social motivation. The results of this chapter are therefore in agreement with the findings in chapter 4, that birds performing more SFP may be more socially motivated. When the results from chapters 4 and 5 are considered together, it appears that SFP causes increased fearfulness in victims, rather than fearfulness predisposing birds to perform SFP.

Chapter 6 compares feather-eating between six SFP pens and six non-SFP pens (n = 12 pens, 600 hens). SFP was determined by feather-scoring and *in situ* behaviour observations. A second experiment in chapter 6 investigates the temporal relationship between feather-eating and SFP in

16 pens (n = 800 hens) to determine whether feather-eating may predict SFP. This was done by assessing feather-eating prior to the development of SFP, at 15 weeks of age, and again after SFP was apparent, at 40 weeks of age. In the first experiment, there was a clear relationship between SFP and feather-eating, where birds in pens with plumage damage had a higher probability of feather ingestion ($P = 0.02$), and showed shorter latencies to peck at, and ingest loose feathers ($P < 0.001$). Birds also ingested feathers from some birds faster than others ($P < 0.001$). In the second experiment, birds tended to peck feathers taken from the rump more times than feathers sourced from the back at 40 weeks of age ($P = 0.06$). These findings demonstrate that birds may preferentially ingest feathers from some birds and particular body areas. This was in line with anecdotal observations of plumage damage occurring mostly on the rump area. Birds pecked at the feathers more ($P < 0.001$), had a higher probability of ingesting feathers ($P < 0.001$), and pecked and ingested feathers more quickly ($P < 0.001$) at 40 than 15 weeks of age. This suggests that birds had a heightened interest in ingesting feathers once SFP was occurring at an older age, although a predictive relationship was not determined due to a lack of variation in plumage damage between pens in this experiment.

Chapter 7 investigates the relationships between fearfulness, plumage damage, and range use. Two pens of 50 hens (n = 100) were fitted with Radio Frequency Identification transponders at 26 weeks of age. The hens’ use of the outdoor range was then tracked continuously over a period of 13 days. Two subpopulations were then identified at the end of the trial: high range users (n = 15) and low range users (n = 15), based on their total time spent outside. These birds were subjected to TI tests and feather-scored at 29 weeks of age. There was no relationship between plumage damage and time spent outside on the range ($P = 0.68$), but there was a negative association between TI duration and range use ($P = 0.01$). This negative association suggests that fearful birds are less likely to use an outdoor range area. Chapter 7 indicates that Radio Frequency Identification technology provides a potential means for quantifying range use in laying hens, on which there is currently very little information.

This thesis supports a relationship between fearfulness and SFP (chapter 5). SFP appeared to cause higher levels of fearfulness in the recipients, rather than fearfulness predisposing birds to perform SFP (chapters 4 and 5). Fearfulness was also negatively associated with time spent on the outside range area (chapter 7). While previous studies have found range use and plumage damage to be inversely correlated, this was not found in chapter 7. The relationships between fearfulness, SFP, and range use warrant further research, particularly in larger flock sizes. Social motivation appears to be implicated in the expression of SFP. Birds that performed more SFP (chapters 3 and 5) also demonstrated higher levels of social motivation in the OFTs (chapters 4 and 5). GFP did not predict plumage damage later in life (chapter 3), but was positively associated with SFP (chapter 5). Results from chapters 3 and 5 do not support the hypothesis that SFP is redirected ground-pecking.

BT significantly reduced plumage damage. While there were no effects of EE on plumage damage, it appeared to enhance the behavioural repertoire during the period in which it was provided (chapter 3), and tended to reduce SFP received in the laying period (chapter 5). Feather-eating may play an important role in SFP (chapter 6), although investigation of a predictive relationship was precluded, and future research should focus on elucidating the relationship between feather-eating and SFP.

This thesis provides information on:

- The short and longer term effects of EE and its interaction with BT. This knowledge could contribute to the development of appropriate husbandry and housing conditions for laying hens to minimise the expression of SFP.
- Feather-eating and its positive association with SFP. It is suggested that birds may have preferences for feathers from particular body areas and birds.
- Behavioural characteristics of pullets prior to the development of high rates of SFP and plumage damage, the usefulness of behavioural tests as predictors of SFP, and the congruency between two behavioural tests, the OFT and the TI test.
- Behavioural traits in birds expressing SFP compared with victimised birds. This information is important in developing standardised phenotypic characteristics of birds that perform SFP. Associated phenotypic characteristics may be utilised in breeding programs to control SFP on a broader scale.
- Range use, fearfulness, and plumage damage. Little is known about range use, why birds access the range area differentially within a flock, and how it may be related to SFP. Information in this area is therefore important to contribute to the understanding of laying hen behaviour and how best to manage and house laying hen flocks.

Therefore, this thesis was successful in 1) increasing the understanding of why SFP occurs; 2) providing information on some of the underlying motivations of birds that perform SFP, 3) providing information on some potential methods for control, and 4) identifying avenues for further research.

CHAPTER 1: General Introduction

GENERAL INTRODUCTION

Severe feather-pecking (SFP) is an injurious behaviour whereby birds peck at, and pull out the feathers of other birds (Savory, 1995). It has been identified as one of the most obvious and serious welfare problems in laying hens (Bilčík and Keeling, 1999, Bestman *et al.*, 2009), and is a significant challenge for the poultry industry worldwide (Nordquist *et al.*, 2011). SFP is linked with elevated stress levels (El-Lethey *et al.*, 2000), and negative welfare states in both the recipient and the bird performing the pecking (Gentle and Hunter, 1990, El-Lethey *et al.*, 2001). The pulling out of feathers is painful for the recipients (Gentle and Hunter, 1990), which then face a higher risk of receiving further SFP (McAdie and Keeling, 2000). SFP can lead to feather damage, extensive feather loss, wounds, cannibalism and death (Savory, 1995). Cannibalism may be described as the ‘pecking and tearing of the skin and underlying tissues of another bird’, and can often result in death (Mench, 2009). Essentially, SFP is a significant welfare concern due to three main reasons:

1. The experience of pain (Gentle and Hunter, 1990) and fear (Hughes and Duncan, 1972) that the recipients of SFP may be subjected to.

2. Subsequent injury due to the feather loss incurred by SFP (Savory, 1995).

3. The occurrence of cannibalism as an indirect result of SFP, and subsequent mortality (Allen and Perry, 1975).

As well as resulting in diminished welfare, SFP also has a negative impact on productivity (Leeson and Morrison, 1978). Even if SFP does not result in mortality, de-feathered birds lose heat faster, particularly in cool climates. These birds therefore have higher energy requirements, resulting in decreased feed conversion efficiency and increased feed costs (Leeson and Morrison, 1978, Tauson and Svensson, 1980). Productivity is further diminished by lower egg production and the incidence of mortality (Peguri and Coon, 1993, Green *et al.*, 2000). Feather-scores and egg production have been documented as being positively correlated, with better feather-scores associated with lower feed intake and higher egg weights (Yamak and Sarica, 2012). Producers may also choose to cull flocks prematurely in order to avoid the costs associated with increased feed requirements and mortality (Green *et al.*, 2000). Hence when flocks experience FP, it results in a less efficient, more expensive operation for the producer.

The reported prevalence of SFP is variable (Nicol *et al.*, 2013). In one study, 57% of farmers of non-cage systems reported that SFP occurred in their last flock (Green *et al.,* 2000). A subsequent study found that 52% of non-cage, organic flocks exhibited severe damage due to SFP (Bestman and Wagenaar, 2003). However, Lambton *et al.* (2010) reported that SFP was observed in 86% of free-range and organic flocks. Since SFP is highly prevalent in commercial settings and contributes to both elevated stress levels and diminished welfare states, preventative and remedial actions should be taken (Bestman *et al.*, 2009).

The incidence of SFP has been correlated with a number of different contributing factors, and is therefore considered to be multifactorial (Lambton *et al.*, 2010, Rodenburg *et al.*, 2013, de Haas *et al.*, 2014a, 2014b). Influential variables include biological, neurological, social, environmental, and genetic (van Zeeland *et al.*, 2009, Rodenburg *et al.*, 2013). Consequently, the root of the behaviour is difficult to identify, and there is ongoing debate regarding the causes (Rodenburg *et al.*, 2008).

TYPES OF BIRD-TO-BIRD PECKING

SFP is not the only form of bird-to-bird pecking that may occur. The various ways in which birds peck at each other derive from different causes, and result in different outcomes. It is therefore important to distinguish between the different forms of pecking in a consistent and reliable manner (Newberry *et al.*, 2007, Rodenburg *et al.*, 2013). Savory (1995) developed a classification system which categorises five types of bird-to-bird pecking which are summarised as follows:

1. Aggressive pecking, which is aimed at the head and neck. It is forceful, and usually performed by dominant birds to subordinates, to establish and maintain dominance within a social hierarchy.
2. Gentle feather-pecking (GFP) is not delivered with force, and does not cause damage to the recipient. The plumage is pecked at, without the removal of feathers. GFP is sometimes directed at non-feather particles on the plumage or around the beak, such as pieces of litter or food. It may be subdivided to three categories:
    - ‘Normal’ pecking at a low frequency,
    - Stereotypic pecking, characterised by high frequency, and
    - Pecking at particles on the plumage.
3. SFP is delivered forcefully and can cause damage to the plumage, feather loss, and pain. Feathers may be grasped and firmly pulled, sometimes resulting in feather removal.

4. Tissue pecking is directed at denuded areas, and can occur as a result of SFP. Tissue pecking may be delivered forcefully and repetitively, resulting in hemorrhage, injuries, and can lead to death of the recipient due to severe injury. Tissue pecking and SFP may grade into each other, with some SFP measurement systems including wounds to the skin as a feather-score.
5. Vent pecking is directed to the cloacal region and is usually investigatory in nature. However, it can escalate and result in death of the recipient bird due to injuries and blood loss (Savory, 1995).

Only GFP and SFP are considered to be feather-pecking (FP) (van Zeeland *et al.*, 2009), and FP is generally divided into those two categories. SFP is thought to be an abnormal behaviour (McAdie and Keeling, 2000), and has been used as an animal model for obsessive compulsive disorder in humans. It has also been compared to barbering in mice, and trichotillomania in humans (Garner *et al.*, 2004, Kops *et al.*, 2013). Some consider GFP to be a normal behaviour which plays a role in social exploration in chicks (McAdie and Keeling, 2002). However, as outlined above, it has been suggested that types of GFP may be stereotypic, and represent a welfare problem (McAdie and Keeling, 2002, Lambton *et al.*, 2010). It is also unclear whether GFP leads to SFP (Rodenburg *et al.*, 2013).

Since GFP and SFP may derive from different motivations, it is important to obtain information on their causes. However, previous studies have not always differentiated between the different forms of FP when conducting observations of bird behaviour (Rodenburg *et al.*, 2013). Studies are needed to elucidate the causes of the behaviours and the roles they play in the behavioural repertoires of laying hens. The present thesis focuses on SFP and some of the potential causative, or associated factors. In addition, the relationship between GFP and SFP is investigated.

HOUSING SYSTEMS

SFP has been documented in all types of commercial housing systems including conventional cages, modified cages, deep litter, free-range, and aviary (Appleby and Hughes, 1991, Huber-Eicher and Sebo, 2001, Bestman *et al.*, 2009). However, it has been more commonly reported as a problem where birds are housed in large group sizes. Hence, group size is thought to affect the risk of SFP, and housing birds in large groups may contribute to an increase in the prevalence of SFP (Hughes and Gentle, 1995, McAdie and Keeling, 2000, Potzsch *et al.*, 2001). As a result, despite SFP existing as a problem in all housing systems, it is a particular concern for birds housed in non-cage systems, where birds are typically housed in larger group sizes (McAdie and Keeling, 2000, Albentosa *et al.*, 2003). Due to the current trend towards non-cage housing systems and in particular, free-range systems, there is a need to address the problems associated with these systems.

BEAK-TRIMMING

Beak-trimming (BT) is a procedure that involves partial removal of the beak (Gentle and Mckeegan, 2007), and is one of the most common methods utilised by the poultry industry to control and minimise the incidence of SFP (Petek and Mckinstry, 2010). However, although BT is widely used, the costs versus benefits are complex. While relatively effective in preventing damage caused by SFP (Lambton *et al.*, 2011), BT is an invasive procedure, and is heavily criticised from a welfare perspective (Gentle, 1986, Freire *et al.* 2011). A ban currently exists in countries such as Norway, Sweden and Finland, with heavy regulation and impending bans in others, including Austria, Belgium, Denmark, Germany, the Netherlands, and the UK (Van Horne and Achterbosch, 2008, Petek and Mckinstry, 2010).

BT stimulates nociceptors in the beak which leads to acute pain during the procedure (Breward and Molony, 1984, Breward and Gentle, 1985, Glatz, 1987), chronic pain in the stump of the beak if performed on older birds (Breward and Gentle, 1985, Gentle, 1986, Gentle *et al.*, 1990, Gentle, 2011), and a reduction in feed intake (Glatz, 1987). It can also cause problems in younger birds, due to the rapid growth and the small size of the beaks; if too much of the beak is removed during trimming it may lead to feeding problems and an increase in mortality. If too little is removed, the beak can re-grow rapidly, and the effectiveness in minimising SFP is reduced.

As well as being criticised from a welfare perspective, there has also been some controversy in the effectiveness of BT and the effects on the bird. Most studies have reported a decrease in cannibalism (Hartini *et al.*, 2002) and plumage damage (Blokhuis and Van Der Haar, 1989, Bolhuis *et al.*, 2009). Hughes and Michie (1982) found significantly lower levels of plumage damage in birds that had undergone BT at 18 weeks of age compared to birds with untrimmed beaks. They concluded that BT is an important procedure to reduce plumage loss, with subsequent beak re-growth being irrelevant. However, some have found that BT does not change pecking preferences, nor the frequency of SFP (Blokhuis and Van Der Haar, 1989, Freire and Cowling, 2012). In a study of 25 free-range farms, Whay *et al.* (2007) found no effect of the extent of BT on SFP, body condition or feather loss at 70 weeks of age. It is currently unknown exactly how BT affects SFP. Possible explanations include 1) learned inhibition from trimming the beak, 2) incomplete sensory feedback affecting sensory perception (Hughes and Michie, 1982), and 3) a chronic pain state resulting in less SFP (Breward and Gentle, 1985, Gentle *et al.* 1986). There is a need for research to investigate the effects of BT, the need for the procedure, as well as potential alternatives to BT.

FORAGING BEHAVIOUR AND THE RE-DIRECTED PECKING HYPOTHESIS

Environmental factors play important contributing roles when exploring the aetiology of SFP in laying hens. Many studies have suggested that SFP develops due to the inability of birds to satisfactorily express foraging behaviour (Blokhuis, 1986, Huber-Eicher and Wechsler, 1997, Dixon *et al.*, 2008, 2010). This behavioural inhibition is thought to cause pecking that would normally be directed at the ground or various foraging substrates, to instead be directed at the feathers of conspecifics, termed ‘redirected’ pecking (Blokhuis and Arkes, 1984). Chickens are known to exhibit extensive voluntary exploratory behaviours and actively seek stimulation (Keeling *et al.*, 2002). This was investigated by Nicol and Guilford (1991) who found that hens which were housed in pens without peat spent more time exploring an empty area near the home pen than those with access to peat.

Providing environmental enrichment (EE) such as manipulable objects may increase the stimulus value of the housing environment and provide an opportunity for the expression of exploratory and foraging behaviours. EE has been reported to enhance the behavioural repertoire and decrease detrimental behaviours such as SFP (McAdie *et al.* 2005). However, studies on EE have yielded inconsistent results (Johnsen *et al.*, 1998, Jones *et al.*, 2002, De Jong *et al.*, 2013). In addition, some studies have not supported the theory of redirected pecking, and instead suggested a hyperactivity model for SFP, whereby birds which perform more SFP also perform more ground-pecking (Newberry *et al.*, 2007, Kjaer *et al.*, 2015). This is discussed more extensively in chapter 2.

REARING PERIOD

Studies have shown that rearing circumstances are important for the development of SFP later in life (Johnsen *et al.*, 1998, de Haas *et al.*, 2014a, b, Janczak and Riber, 2015). A review by Rodenburg *et al.* (2008) reported that conditions in early-life (0-7 weeks of age) have large effects on behavioural development, including SFP. Nørgaard-Nielsen *et al.* (1993) also concluded that the environment during rearing is important, and it is likely that an imprinting process occurs that continues to affect birds for the remainder of their lives. These results correlate with the findings of other studies such as those by Blokhuis and Van Der Haar (1992) who demonstrated that providing grain in the litter during the rearing period reduced plumage damage during the laying period. Vestergaard *et al.* (1993) also found that substrate during rearing has a reductive effect on SFP later in life (i.e. providing a substrate such as sand, straw or wood shavings when compared with no substrate).

Bestman *et al.* (2009) found that when pullets did not exhibit feather damage due to SFP during rearing, in 71% of the cases, they did not show SFP damage in the laying period either. When feather damage was evident during rearing, in 90% of the cases it was also expressed during the laying period. Bright (2009) suggested that when investigating SFP as it relates to environment and management, future studies would benefit from focusing on the rearing period. There is a current need to identify forms of enrichment that are attractive to chicks, pullets and hens over a long period of time, and effectively reduce SFP. Further studies are required to investigate the effects of EE during early-life on SFP performed later in life. This is discussed further in chapter 2.

FEARFULNESS AND COPING STYLE

Animals have particular behavioural, neuroendocrinological, and physiological responses, or ‘coping styles’, to stressful or threatening stimuli (Dantzer and Mormede, 1983, Jones and Satterlee, 1996, Korte *et al.*, 1997). Coping styles are typically described as either active (i.e. fight or flight) or passive (conservation or withdrawal). Differences in coping styles have been found to exist between individual animals within a population (Jensen *et al.*, 2005). Coping style is thought to be linked with the expression of SFP (de Haas *et al.*, 2010), which may be measured in behavioural tests. Additionally, when behavioural tests are performed during the rearing period, the responses may be indicative of SFP behaviour when older (Rodenburg *et al.*, 2004).

Fearfulness is thought to play an important role in the expression of SFP (Rodenburg *et al.* 2013). Fearfulness, coping style and social motivation have been estimated in laying hens in previous experiments by well-validated behavioural tests; the open-field test (OFT) and the tonic immobility (TI) test (Forkman *et al.*, 2007). TI is described as an unlearned reaction, induced by a brief period of physical restraint, and is characterised by a catatonic state and reduced responsiveness to stimulation. The duration of time spent in the state of TI is positively related to fearfulness, is relatively unambiguous (Jones *et al.*, 1995), and has long been used as a method of estimating fearfulness (Gallup Jr, 1979, Jones, 1986). The OFT involves the introduction of an individual subject to a novel arena. The test is well-validated, although birds' reactions to the OFT may reflect both fear, as well as the motivation for social reinstatement in response to social isolation (Forkman *et al.*, 2007).

Fearful chicks may be more likely to develop SFP later in life (Rodenburg *et al.*, 2004). SFP may also cause higher levels of fearfulness in victimised birds (Hughes and Duncan, 1972, Rodenburg *et al.*, 2010). The causal relationship is not determined, and the relationship between fearfulness and SFP is not well-understood (de Haas *et al.*, 2010). Therefore, uncovering links between behavioural responses in tests during rearing, and the development of SFP later in life is important in developing a greater understanding of SFP and its underlying behavioural causes. Information pertaining to behaviour in rearing also has potential to contribute to genetic selection programs, which aim to select birds less likely to express SFP.

FEATHER-EATING

The incidence of feather-eating has been associated with the occurrence of SFP (Harlander-Matauschek *et al.*, 2006a), and birds identified as feather-peckers have exhibited a higher interest in feather-eating than low- or non-SFP hens (Kjaer and Bessei, 2013). Feathers are thought to offer similar digestive function to that of insoluble fibre, acting to increase the passage of digesta (Harlander-Matauschek *et al.*, 2006b). Nevertheless, birds have expressed preferences for feathers in a choice test, when both fibre and feathers were available (Kjaer and Bessei, 2013), demonstrating a specific preference for feathers. It has been suggested that the development of

SFP is linked to the diet. The hypothalamus is the modulator of feeding behaviour, and may play a role in the development and regulation of behaviours related to SFP (Richards and Proszkowiec-Weglarz, 2007, Brunberg *et al.*, 2011).

Feather-eating may be affected by the properties of feathers. Feathers possess olfactory, visual, gustatory, and tactile qualities which may be attractive to birds (Mckeegan and Savory, 2001, Harlander-Matauschek and Feise, 2009, Harlander-Matauschek *et al.*, 2010). Feathers may also offer material for exploration, and be considered as foraging material (Harlander-Matauschek and Bessei, 2005). While the relationship between feather-eating and SFP appears to be important, the specific appetite that birds show for feathers is not understood. In addition, certain birds and certain body areas can sometimes be targeted for SFP and exhibit extensive feather loss, while others remain undamaged. It is not known why this occurs, and if the properties of the feathers contribute to this. An important aspect of feather-eating is to determine whether it may predict SFP, as this is currently unknown.

RANGE USE

Studies suggest a negative correlation between range use and SFP in free-range systems (Green *et al.*, 2000, Bestman and Wagenaar, 2003, Mahboub *et al.*, 2004, Lambton *et al.*, 2010). However, little is known about ranging behaviour in free-range laying hens, and why some birds may use the range area more than others. Range use is affected by a number of factors. In particular, fearfulness may affect birds' propensity to access the range area, where more fearful birds may be less likely to venture outside (Grigor *et al.*, 1995). However, very few studies have investigated the relationship between fearfulness, range use, and SFP. Additionally, most studies on range use have collected data through the use of questionnaires and surveys to estimate range use (Nicol *et al.*, 2003, Lambton *et al.*, 2010). Questionnaires present obvious limitations, which include the inability to monitor variation in range use throughout the day, and between days.

It has been identified that there is a need for more empirical data on range use, and some studies have utilised technologies such as Radio Frequency Identification to collect data (Mahboub *et al.*, 2004, Richards *et al.*, 2011, Durali *et al.*, 2014). Radio Frequency Identification technology is currently emerging as an application with the potential to collect large amounts of data on bird behaviour in free-range systems. With the increased prevalence of free-range systems in countries including Australia (Rault *et al.*, 2013), there is a need to understand the effects of this type of housing system on parameters related to bird welfare.

INDIVIDUAL BIRD VARIATION

Differences on an individual level are important in the expression of SFP (Hughes and Duncan, 1972, Brunberg *et al.*, 2011, Daigle *et al.*, 2015). As outlined, the causal factors driving the development of SFP are undetermined, and some research has investigated individual differences between birds (Jensen *et al.*, 2005, Daigle *et al.*, 2015). Jensen *et al.* (2005) explored phenotypic relationships between SFP and responses to various behavioural tests, where birds identified as feather-peckers exhibited higher activity levels in stressful situations. By investigating behavioural traits linked to the propensity to perform SFP, quantitative trait loci may be identified. Genetic selection may offer a way forward in addressing, and reducing the expression of SFP (Albentosa *et al.*, 2003, Rodenburg *et al.*, 2004, Bessei and Kjaer, 2015). It is thought that selection against SFP will most likely not have negative effects on production related traits, although more information is required in this area.

There is sufficient genetic variation to select against SFP (Kjaer *et al.*, 2001). However, SFP occurs in short, unpredictable bouts (Bessei *et al.*, 1999). This makes data collection on individual birds time-consuming and difficult in commercial situations (Bessei and Kjaer, 2015). Selection traits have not been standardised, and genetic selection has not been successful thus far (Bessei, 1984, Bessei and Kjaer, 2015). The measurement of SFP, and the collection of data under standardised conditions are therefore important, and the ability to identify ‘high’ feather-peckers with phenotypic, behavioural traits may be useful in breeding programs (Albentosa *et al.*, 2003).

More information is required on the consistency of particular behavioural tests between individual birds as well as between different ages. In addition to this, it is not clear whether individuals can be categorised into distinct behavioural ‘types’ (Albentosa *et al.*, 2003). Identifying associations between high and low SFP birds and other behavioural characteristics may also help explain why some birds are more predisposed to feather-peck.

AIMS OF THE THESIS

This thesis investigates several factors which are implicated in the expression of SFP. The first two experimental chapters (3 and 4) assess the impacts of BT and EE. Chapter 3 investigates the impacts of BT and EE on the behavioural repertoire of pullets during the rearing period, and the subsequent effects on plumage damage later in life. Chapter 4 investigates the impacts of EE and

BT on fearfulness and coping style in a TI test and OFT during rearing and how these tests may be used as predictors of SFP later in life. Chapter 5 categorises individual birds as those performing SFP compared with those receiving SFP, in order to study associated behavioural traits; fearfulness and coping style. Chapter 6 investigates feather-eating in pens of birds expressing SFP compared with those expressing minimal SFP, and the predictive relationship between feather-eating and SFP, while chapter 7 investigates the associations between fearfulness, range use, and plumage damage.

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CHAPTER 2: Severe feather-pecking in non-cage laying hens and some associated and predisposing factors: a review.

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Published in: *World's Poultry Science Journal* (2016)

72:103-114

OVERVIEW OF CHAPTER 2

Severe feather-pecking is a complex, multifactorial behaviour which exists as a highly detrimental, extremely prevalent behavioural problem in laying hens worldwide. There is a need to improve our knowledge about why severe feather-pecking occurs, and identify areas for future research. Chapter 2 provides a critical review of areas of research relevant to this thesis, on topics including foraging behaviour, feather-eating and individual bird variation. This review provides context for the thesis, identifies potential gaps in the literature and avenues for future research on severe feather-pecking.

ABSTRACT

Severe feather-pecking (SFP), whereby birds vigorously peck at, and pull out the feathers of conspecifics, is an extremely injurious, highly prevalent behaviour. It has acute negative impacts on the egg industry worldwide, in terms of both animal welfare and production efficiency. A large volume of research has been conducted to investigate the underlying causes. However, although many contributing factors have been identified, it remains largely unpredictable, and very difficult to control. Environmental influences during the rearing period, feeding behaviour, feather-eating and individual bird differences play important roles in the expression of SFP. This paper provides a review of some of the literature on the aforementioned causes of SFP in laying hens.

Keywords: laying hen, feather-pecking, environmental enrichment, foraging, rearing, feather-eating

INTRODUCTION

Severe feather-pecking (SFP) is defined as a detrimental behaviour, whereby birds vigorously peck at, and pull out the feathers of other birds (Savory, 1995). It has been identified as one of the most significant risks to animal welfare in the egg industry (Bestman *et al.*, 2009). Considered to be a multi-factorial behaviour, SFP has many influential factors including early-life experiences (Janczak and Riber, 2015), the environment (Rodenburg *et al.*, 2013), genetics (Jensen *et al.*, 2005, Rodenburg *et al.*, 2008), fearfulness (Rodenburg *et al.*, 2004, Uitdehaag *et al.*, 2009), diet and feeding behaviour (Kjaer and Bessei, 2013), and individual bird variation (Bessei and Kjaer, 2015, Daigle *et al.*, 2015). Despite extensive research, the underlying mechanism(s) and developmental causes of SFP are not fully understood, and it is often unpredictable in commercial situations (Bessei and Kjaer, 2015).

Currently, SFP is managed largely by remedial measures such as reduced ambient lighting and beak-trimming (Petek and McKinstry, 2010), which attenuate, but do not address the cause of the behaviour. Beak-trimming, whereby the tip of the beak is removed, is a controversial practice which is banned in countries such as Switzerland, Sweden, Norway and Finland, and is due to be banned in others, including the UK, Denmark, Germany and The Netherlands (Gilani *et al.*, 2013, de Haas *et al.*, 2014a, Janczak and Riber, 2015).

Many studies have suggested that SFP develops as a redirected behaviour, due to the inability to satisfactorily express foraging behaviours (Blokhuis and Arkes, 1984, Blokhuis, 1986, Huber-Eicher and Wechsler, 1997). Environmental enrichment (EE) has been reported to enhance the behavioural repertoire, and decrease injurious behaviours such as SFP (McAdie *et al.*, 2005, Rodenburg *et al.*, 2013). However, studies assessing the impact of EE in the housing of domestic fowl and its effect on SFP have yielded equivocal results (Jones *et al.*, 2002, De Jong *et al.*, 2013, Bessei and Kjaer, 2015).

Feeding behaviour has also been implicated in the expression of SFP, and a positive relationship has been found between feather-eating and SFP (Mckeegan and Savory, 1999, Harlander-Matauschek *et al*., 2006a). Further, some have suggested that feather-eating may be the prime causal factor for the expression of SFP (Bessei, 2011, Bessei and Kjaer, 2015). However, more research is required to investigate the specific role of feather-eating in SFP. Research investigating EE and the rearing period, individual bird variation, and feather-eating is at the forefront of the efforts aiming to identify the underlying mechanisms and causal factors implicated in the expression of SFP. This review aims to focus on these research topics in a critical analysis.

GENTLE FEATHER-PECKING

There are two types of feather-pecking; gentle and severe. They are thought to be distinct behaviours, with different underlying neural mechanisms and motivations (Hughes and Buitenhuis, 2010). As well as deriving from different causes, they result in very different outcomes, where gentle feather-pecking does not cause damage to the recipient. The relationship between gentle and SFP and their causes are not fully understood. There is, therefore, a need to distinguish between the two types of feather-pecking in research in order to gain an improved understanding of feather-pecking, facilitate accurate data collection, and also to ascertain whether gentle feather-pecking may be a precursor to SFP (Rodenburg *et al.*, 2013).

THE RE-DIRECTED PECKING THEORY

The dominant hypothesis to explain the behavioural cause of SFP is that the pecking is misdirected from the environment. Foraging behaviour consists of two main components; food-searching (the appetitive phase) and food consumption (the consummatory phase). In poultry, although feed may be provided *ad libitum*, thereby satisfying the motivation for the consummatory phase, birds still exhibit food-searching behaviours, such as ground-pecking and ground-scratching, where the appetitive phase has not been fulfilled (Keeling, 2002). Where food-searching behaviours are inhibited, ground-pecking may be replaced with SFP (Blokhuis, 1986, Huber-Eicher and Wechsler, 1998). In support of this theory, Dixon *et al.* (2008) found similar motor patterns between foraging pecks and pecks associated with SFP. This indicates similar morphologies between the two types of pecking, and that SFP may result from frustrated motivations to forage.

While the theory of redirected pecking is still relevant (Rodenburg *et al.*, 2013), Newberry *et al.* (2007) found that birds which were more likely to perform SFP as adults, were those that exhibited more foraging as chicks. Hartcher *et al.* (2015) also reported that groups of birds with more plumage damage at 43 weeks of age had exhibited more ground-pecking between 3 and 14 weeks of age. In addition, these birds performed more ground-pecking and SFP during rearing than birds which had less plumage damage at 43 weeks of age. Similarly, Bilčík and Keeling (2000) found a positive correlation between SFP and ground-pecking during the laying period.

Channing *et al.* (1998) found that birds characterised as feather-peckers, pecked at inanimate objects sooner and more than birds characterised as non-peckers. These findings suggest that active birds may be more likely to perform more pecking behaviours, both at other birds and the environment. In line with this, Kjaer (2009) found that birds from a genetic line which had been selected for high levels of SFP (Kjaer *et al*., 2001) had higher locomotor activity than birds from a line selected for low levels of SFP, and suggested a hyperactivity disorder model for SFP. The hyperactivity model suggests that birds performing more SFP are also those which are more active. Kjaer *et al.* (2015) subsequently investigated recurrent perseveration in the lines which had been selected for high and low SFP. Recurrent perseveration is the tendency for an animal to repeat responses in an inappropriate manner, and is related to stereotypic behaviour (Kjaer *et al.*, 2015). This experiment was conducted because SFP is thought to be a persistent, compulsive behaviour (Kops *et al.*, 2013). Kjaer *et al.* (2015) found that birds selected for SFP showed lower recurrent preservation behaviour. The hyperactivity model of SFP was supported.

So, while many previous studies have agreed with the redirection hypothesis (Blokhuis, 1986, Blokhuis and van der Haar, 1992), another theory has emerged, which suggests a positive association between SFP and ground-pecking (Hansen, 1994, Bilčík and Keeling, 2000, Newberry *et al.*, 2007). These two theories may not be conflicting (Rodenburg *et al.*, 2008); individual birds may have a higher motivation to perform pecking behaviours in the form of ground-pecking and SFP. However, on a group level, birds may still direct proportionally more of these pecks to the environment than the feathers of conspecifics, if they are provided with an environment in which foraging is stimulated (Rodenburg *et al.*, 2008, 2013).

THE REARING ENVIRONMENT AND ENVIRONMENTAL ENRICHMENT

In contrast to non-captive or ‘wild’ environments, in which there is, inherently, a variety of ever-changing sensory stimuli, captive environments offer relatively fewer opportunities for stimulation (Wells, 2009). Since the assertion was made that SFP is redirected foraging, the type of EE which may be relevant to study with the aim to reduce SFP, is EE which stimulates foraging behaviour. Indeed, a number of studies have investigated the provision of EE, with the aim to enhance sensory stimuli and foraging behaviours, and reduce the expression of SFP (Rodenburg *et al.*, 2013). Wells (2009) suggested that EE which targets the dominant sense for a particular species would yield the most beneficial outcomes in terms of animal welfare. Despite this, a recurring issue in the provision of EE is the lack of functional relevance of the stimuli to animals, and an improved understanding of behaviour performed in captivity would improve research on EE (Newberry, 1995). However, stimuli which are specific to the natural habitat of the species should not be exclusively considered as beneficial. Indeed, studies have investigated the effectiveness of EE devices which do not occur naturally. The provision of music or the presence of radios has had beneficial effects on a range of species including chickens (Wells, 2009), and has been shown to reduce feather damage in laying hens (de Haas *et al.*, 2014a). However, these effects require further investigation, as others have found radios and specific noise stimuli to increase fearfulness in hens (Campo *et al.*, 2005).

Trials have been conducted to identify practical and effective forms of EE. While one type of EE may be effective in one trial, this does not guarantee consistent effectiveness across all flocks, or conversely, an ineffective treatment may be effective in alternate settings. There is a need to repeatedly trial differing types of EE, to determine which consistently reduce abnormal behaviours (Mason *et al.*, 2007). There have been equivocal findings relating to the provision of EE and the resultant incidence of SFP, some of which are reported in Table 1. It should be kept in mind that there may be a skew in publications on the effects of EE, where studies which report negative results may be less likely to be published.

Table 1. Summary of publications, in chronological order, which investigated environmental factors predominantly designed to stimulate foraging behaviours, and their effect on feather-pecking and plumage damage. Additional effects on behaviours such as foraging or dustbathing are not reported here.

		Measurement		**Outcome**	
Reference(s)	**Age***	**Behaviour observations (week)**	**Plumage damage (week)**	**Reduced feather-pecking**	**Reduced plumage damage**
Hughes and Duncan (1972)#	0		0-21		✓
Blokhuis and Arkes (1984)#	0	0-17		✓	
Blokhuis (1986)#	0	6		✓	
Blokhuis and van der Haar (1989)#	0-18	7-42	17-42	✓	✓
Blokhuis and van der Haar (1992)#	0-17	3-42	17-42	✘	✓
Nørgaard-Nielsen *et al.* (1993)#	0-18 & 18‡		28-72		✓
Nørgaard-Nielsen (1997)#	0-3[†]	~1-6	~6	✘	✓

Wechsler and Huber-Eicher (1998)#	19-30	19-30	30	✓	✘
Johnsen *et al.* (1998)#	0-4‡	5-6 & 40-41	19-45	✓	✓
Huber-Eicher and Wechsler (1998)#	0‡	4-5		✓	
Gunnarsson *et al.* (1999)♣	0-2		33-40		✘
Aerni *et al.* (2000)#	19	19-25	27	✓	✓
McAdie and Keeling (2002)#	0	13-32	12-29	✓	✓
McAdie *et al.* (2005)#	0	~8	35	✓	✓
Hetland *et al.* (2004b)#	0-16		72		✓
Dixon and Duncan (2010)#	0-4[†]	0-4		✓	
Daigle *et al.* (2014)#	22	21-37	21-37	✘	✘
de Haas *et al.* (2014a)♣	17‡		40		✓
de Haas *et al.* (2014b)♣	4	5-10	5-15	✓	✓
Hartcher *et al.* (Submitted, 2015)#	3-20	43-44	43	✓	✘

* Week(s) of age at which environmental enrichment was provided. One number denotes that enrichment was provided from that age onwards. Zero weeks denotes the provision of enrichment from 0, 1 or 2 days of age. Enrichment was pecking stimuli or foraging material including wood shavings, grain, straw, pecking strings etc., compared to a control treatment without access to a foraging substrate or pecking stimuli.

[†] Enrichment was a substrate attractive for dustbathing i.e. peat moss or sand.

‡ A foraging, or pecking stimulus as well as a non-foraging enrichment (e.g. sand) were provided.

# Denotes a study using small group sizes, whereas ♣ denotes a study which was conducted in commercial settings, with larger group size(s).

An aspect to consider in experiments investigating the effects of EE is group size. SFP may not occur to the same extent in small-group, experimental settings as in commercial situations, and EE may also elicit different responses in birds kept in different group sizes. The majority of studies in Table 1 were conducted in small-groups, in experimental situations. These experiments have benefits in that treatment effects may be investigated in controlled settings, with a number of replicates. However, there is a need for further studies to investigate EE in larger group sizes, in commercial or semi-commercial settings. The findings by de Haas *et al.* (2014a, b) suggest that different strains may require different management strategies, due to differing levels of sensitivity to stress and environmental stimuli. Hence the genetic background of the birds should also be taken into consideration when testing EE, and when considering why the effects of EE may vary between flocks.

McAdie *et al.* (2005) found that the provision of pecking strings from one day of age improved plumage condition and reduced SFP at 35 weeks of age. Jones *et al.* (2002) also concluded that bunches of white string are attractive pecking stimuli, but Hartcher *et al*. (2015) found no effect of pecking strings provided from 12 days of age on plumage damage at 43 weeks of age. Nørgaard-Nielsen *et al.* (1993) reported that the provision of sand and peat from one day of age, and straw during the laying period both reduced plumage damage. More recently, a study by de Haas *et al.* (2014a) on commercial farms found that the provision of pecking blocks, round bell drinkers, radios, roosters, good litter quality, and aviary housing compared to floor housing resulted in lower levels of feather damage. However, Wechsler and Huber-Eicher (1998) found that while foraging material, in the form of polystyrene blocks, reduced the rate of feather-pecking, there was no effect on plumage damage. Similarly, Hartcher *et al.* (2015) found no effect of increased litter depth and the provision of whole oats in the litter during rearing on plumage damage later in life.

The rearing environment and early life experiences play important roles in the development of SFP (Rodenburg *et al.*, 2013, de Haas *et al.*, 2014b, Janczak and Riber, 2015). Complexity in the rearing environment may reduce fearfulness and SFP later in life (de Haas *et al.*, 2014b, Janczak and Riber, 2015). Some studies have suggested that the first 10 days of a bird's life may be a critical period in which EE should be provided in order for birds to learn to interact with the EE (Sanotra *et al.*, 1995, Huber-Eicher and Wechsler, 1997, McAdie and Keeling, 2002, Janczak and Riber, 2015). Indeed, Johnsen *et al.* (1998) found that the substrate in the first four weeks of life had a major influence on SFP and plumage damage later in life. Nørgaard-Nielsen (1997) found that the presence of sand in the first 21 days of life reduced plumage deterioration, while there was no effect on SFP when sand was provided from 41 days of age. Conversely, Gunnarsson *et al.* (1999) did not find that access to litter in the first two weeks of life affected plumage damage later in life. Similarly, De Jong *et al.* (2013) found no effect of the provision of substrate in the first 21 days of life on feather-scores at 40 weeks of age, although all rates of SFP and incidence of plumage damage were low in the experiment. Dixon and Duncan (2010) found a reduction in SFP when chicks had access to peat moss in the first four weeks of life, but did not find a correlation between substrate availability during the first two weeks of life and subsequent SFP at three and four weeks of age.

While it has been suggested that the rearing period, and particularly the first 10 days of life are important, the long-term effects of particular types of enrichment provided at different ages has yet to be determined. The current substrate is also of great importance (Blokhuis and Arkes, 1984, Nicol *et al.*, 2001, Rodenburg *et al.*, 2013, Janczak and Riber, 2015). Research is needed to identify the effects of particular types of enrichment, and the best age at which to provide it in order to prevent subsequent SFP. Various aspects and different forms of EE should be investigated (Tarou and Bashaw, 2007). For example, Sambrook and Buchanan-Smith (1997) suggested that controllability of an enrichment device by animals may be more important than the device's complexity. Huber-Eicher and Wechsler (1998) found that the quality and availability of foraging materials has a significant effect, and de Haas *et al.* (2014b) found that litter restriction around 5 weeks of age led to high levels of SFP. Key aspects to consider in the provision of EE are: the age at which it is provided, the group size to which it is provided, the type and form in which it is provided, the availability throughout the lives of the birds as well as on a daily basis, the effects on the behavioural repertoire and SFP, the practical feasibility of installing and managing EE in commercial settings, and ultimately, whether there are consistent effects of particular types of EE on bird behaviour.

FEEDING BEHAVIOUR AND FEATHER-EATING

Redirected pecking prevails as the dominant theory to explain the cause of SFP. However, SFP also occurs in environments which appear to provide adequate stimuli. This has led to the suggestion that feather-eating may be the primary motivation driving the expression of SFP (Harlander-Matauschek *et al*., 2006a, Bessei, 2011, Bessei and Kjaer, 2015). Ethology and nutrition are both important to consider when addressing SFP, and theories relating to redirected foraging behaviour, and nutritional deficiencies and feather-eating may not be mutually exclusive. Dietary deficiencies can stimulate exploratory behaviour, which may result in redirected pecking and hence the occurrence of SFP (Kjaer and Bessei, 2013). In addition to this, feather-eating may represent a form of redirected pecking. McKeegan and Savory (1999) concluded that if feather-eating becomes established, a low availability of feathers on the floor may incite SFP.

Levels of insoluble fibre in the diet are thought to affect SFP (Hetland *et al.*, 2004a), and feather-eating may increase in the absence of structural components or 'roughage' in the feed (Hetland *et al.*, 2005, Kalmendal and Bessei, 2012). Feathers increase the speed of feed passage, similar to the effects of insoluble fibre (Harlander-Matauschek *et al.*, 2006b), and Kriegseis *et al.* (2012) found that the inclusion of 10% feathers in the diet reduced the amount of SFP and improved plumage condition. Birds from high SFP lines show significantly more feather-eating than birds from low SFP lines (Harlander-Matauschek and Bessei, 2005, Harlander-Matauschek *et al.*, 2007, Harlander-Matauschek and Häusler, 2009), and work harder for feathers (Harlander-Matauschek *et al.*, 2006a). Additionally, birds selected for high SFP (Kjaer *et al.*, 2001) differ in their bacterial metabolites and therefore their intestinal microbial metabolism, due to feather degradation in the gut, when compared with birds selected for low SFP activity (Meyer *et al.*, 2013).

Studies have found that olfactory (McKeegan and Savory, 2001), visual (McKeegan and Savory, 1999, McAdie and Keeling, 2000, Bright, 2007), gustatory (Harlander-Matauschek *et al.*, 2008, 2010) and tactile (Harlander-Matauschek and Feise, 2009) cues may play a role in the selection of feathers for consumption. Additionally, feather characteristics may influence the selection of body areas for SFP (Harlander-Matauschek *et al.*, 2007). Feather-eating appears able to be learnt (Ramadan and Von Borell, 2008). However, the temporal relationship between SFP and feather-eating is not clear. De Haas et al. (2014a) found that flocks with severe plumage damage at 40 weeks of age tended to perform more feather-eating during rearing compared with flocks with no severe damage at 40 weeks, although some SFP had also developed during rearing. More information is required in order to understand the relationship between feather-eating and SFP, whether feather-eating may predict SFP, and what feathers offer to birds in terms of their role in exploration, foraging and dietary function. It is important to elucidate the function of feather-eating, and whether it may be nutritionally driven, represent a behavioural need for environmental stimulation, or both.

INDIVIDUAL BIRD VARIATION

While changes to the environment in commercial settings are made on a group level, individual bird variation is important in the expression of SFP (Jensen *et al.*, 2005, Brunberg *et al.*, 2011, Rodenburg *et al.*, 2013). Individual birds' characteristics, such as their levels of fearfulness, social motivation, and ability to cope with stress are implicated in the expression of SFP (Jensen *et al.*, 2005, Rodenburg *et al.*, 2013). Wechsler *et al.* (1998) identified 12% of birds in their experiment as 'high rate peckers', which initiated 39% of all feather-pecking. It was concluded that some individual birds may be characterised as performing higher rates and more severe forms of feather-pecking. Uitdehaag *et al.* (2009) suggested that more fearful birds may be predisposed to becoming victims of SFP, and that there is evidence for two distinct types of birds, those with a low expression of SFP and those that may have a higher susceptibility to develop SFP. In addition, some birds are more responsive to environmental conditions.

Recently, Daigle *et al.* (2015) investigated not only individual behavioural profiles, but also the consistency of birds to remain in those behavioural profiles between 21 and 37 weeks of age. Of the birds identified as severe feather-peckers at 21 weeks of age, 22.5% of these remained as severe feather-peckers, while 53.8% exhibited inconsistent behavioural patterns across the observation periods (during two 30 min periods at 21, 24, 27, 32, and 37 weeks of age). Kops *et al.* (2013) reported differences between behavioural phenotype (severe feather-peckers, victims, and non-peckers) and serotonergic neurotransmission in the dorsal thalamus and striatum, leading to the hypothesis that birds initiating SFP may be more likely to do so due to genetic, neurochemical 'deficits', which may also underlie differences in coping strategies in behavioural tests (Rodenburg *et al.*, 2013).

It has been thought for some time that there is a small number of individuals within a flock that account for the majority of SFP (Savory, 1995). Additionally, it has recently been suggested that there may be 'primary feather-peckers' in a flock with genetic predispositions to perform SFP, which may have an increased appetite for feathers and fibre due to their intestinal microbiota (Meyer *et al.*, 2013, Bessei and Kjaer, 2015). Chickens are able to learn from conspecifics in a number of different ways (Nicol, 2006), and studies have suggested that once established, SFP behaviour may readily spread throughout a group via learning (Zeltner *et al.*, 2000, Rodenburg *et al.*, 2013). Smaller group sizes can therefore constrain the spread of SFP (de Haas *et al.*, 2014a, Bessei and Kjaer, 2015) and may also play a role in mitigating stress (Rodenburg *et al.*, 2013).

Individual and strain differences imply a strong genetic component to SFP (Jones and Hocking, 1999), and it is thought that genetic markers may facilitate the identification of birds that are predisposed to perform SFP. Genetic selection may be required to aid the control of SFP, by eliminating the particular physiological and genetic characteristics associated with SFP from the breeding stock (Savory, 1995, Kjaer and Bessei, 2013, Bessei and Kjaer, 2015). However, more information is required on the potential relationships between SFP and other associated traits (Jones and Hocking, 1999).

CONCLUSIONS

SFP is a complicated behaviour, with a number of influential factors to consider when aiming to control its expression. While the rearing period and EE are thought to be important, the most effective type of EE has yet to be determined. In addition, the full effect of EE on birds is not completely understood. That is, how the rearing environment and different types of EE affect behaviour at the time EE is provided, as well as the effects later in life. Feather-eating is thought to be related to SFP, but little is known about the specific role it plays and whether it may predict SFP. Individual variation between birds is important to consider in all elements of animal behaviour, including the propensity for birds to interact with their environment and other members of the flock, their underlying behavioural characteristics, and their dietary requirements. Understanding the behavioural repertoires of birds on an individual and group level, and how different behaviours interrelate, is of great consequence in determining the most appropriate housing conditions in which to optimise bird welfare, particularly when aiming to reduce a detrimental behaviour such as SFP in commercial settings.

In conclusion, the environment has large effects on bird behaviour. EE should be provided while taking into account housing system, group size, and strain of bird. However, individual bird variation may be overriding, and a particular appetite for feathers may contribute to the occurrence of SFP. The role of feather-eating and individual variation should be understood in order to cater for birds' behavioural and nutritional needs, optimise management, and investigate the potential for genetic selection programs. Therefore, future research in this area should comprise an integrated approach incorporating work on EE, feather-eating, and individual bird variation. Studies need to 1) apply various forms of EE consistently across flocks in commercial and experimental settings to identify EE that meets birds' biological needs, and effectively abates SFP, 2) determine how feather-eating is implicated in the expression of SFP, and 3) investigate whether birds may be categorised due to their involvement in SFP, and whether phenotypic traits may be associated with SFP for future selection programs. An integrated approach has the potential to result in birds with lower genetic predispositions to perform SFP, housed in environments that address their behavioural requirements, thereby reducing the risk of SFP.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support in the form of a postgraduate stipend, awarded to Kate Hartcher by the Poultry Cooperative Research Centre, established and supported under the Australian Government’s Cooperative Research Centres Program. The authors also thank the insightful contributions of an anonymous reviewer.

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CHAPTER 3: The effects of environmental enrichment and beak-trimming during the rearing period on subsequent feather damage due to feather-pecking in laying hens

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Published in: *Poultry Science* (2015)

94:852-859

OVERVIEW OF CHAPTER 3

In the review in chapter 2, avenues for future research were identified. Environmental influences in the rearing period are particularly important in the expression of severe feather-pecking. There is a need for research to investigate different types of environmental enrichment, how they affect bird behaviour at the time period in which they are provided during the rearing period, as well as effects on severe feather-pecking later in life. The review also reiterated that the behavioural repertoires of laying hens must be understood in order to determine methods of control for severe feather-pecking. The relationship between gentle feather-pecking and severe feather-pecking is currently not well-understood. There is a need to ascertain whether gentle feather-pecking may be related to severe feather-pecking, or even be used to predict it. Further, there is currently some contention on whether the re-directed foraging hypothesis is the most accurate explanation for severe feather-pecking, or whether the proposed alternative model, the ‘hyperactivity’ model, may be more relevant. This chapter investigates the effects of certain types of environmental enrichment on the behavioural repertoires of pullets during the life phase in which it is provided, the rearing period, and on plumage damage later in life. This chapter also investigates the relationship between gentle and severe feather-pecking, as well as the redirected foraging hypothesis, and how pecking behaviours and plumage damage are affected by beak-trimming.

ABSTRACT

This experiment investigated effects of environmental enrichment and beak-trimming during the rearing period on behaviour in rearing and plumage damage later in life. Treatments were applied in a 2 × 2 factorial arrangement. Half of the birds were beak-trimmed at 1 d of age using an infra-red laser. A follow-up light-trim was performed at 11 weeks of age with a hot blade. Environmental enrichment consisted of pecking strings, whole oats in the litter, and greater litter depth. Sixteen pens of 50 ISA Brown laying hens were used. Four pullets were selected from each pen as focal birds and observed in their home pens between three and 14 weeks of age. Plumage damage was scored at the end of the experiment in week 43. Beak-trimmed birds performed less ground-pecking ($P = 0.003$), less severe feather-pecking ($P = 0.02$) and more gentle feather-pecking ($P = 0.02$) than their non-trimmed counterparts during the rearing period. These birds also exhibited less feather damage in week 43 ($P < 0.001$). The results indicate that gentle feather-pecking during rearing is not related to plumage damage when older. Additionally, higher rates of ground-pecking and severe feather-pecking during rearing may be predictive of plumage damage later in life. There was no effect of enrichment on plumage damage. It was concluded that while there was no effect on enrichment, beak-trimming appeared to be effective in reducing plumage damage in week 43.

Key words: feather-pecking, rearing, beak-trimming, enrichment, behaviour

INTRODUCTION

Feather-pecking (FP) in laying hens (*Gallus gallus domesticus*) is defined as a non-aggressive behaviour whereby birds peck at or pull out the feathers of conspecifics. Different forms of FP occur including gentle and severe. Severe FP (SFP) involves the forceful pecking at and pulling of feathers and sometimes the removal and ingestion of pulled feathers. It is often injurious in nature and can result in feather damage, feather loss, wounds, pain, and sometimes cannibalistic pecking and death (Gentle and Hunter, 1991; Savory, 1995; Rodenburg *et al*., 2013). SFP is vigorously performed but is distinct from aggressive pecking, which is associated with social behaviour and the establishment and maintenance of dominance within social hierarchies. Aggressive pecking is usually targeted at the head region and does not typically result in plumage damage to the body (Savory, 1995; Gilani *et al*., 2013). Gentle FP (GFP) involves pecking at the plumage of other birds without much force and with no subsequent removal of feathers. It results in little or no damage and the receiver usually does not exhibit an adverse reaction such as vocalisations or a retaliatory peck. Previous studies have found GFP to be a normal behaviour that plays a part in social exploration (Riedstra and Groothuis, 2002) with no association to SFP (Hughes and Buitenhuis, 2010). Others suggest that it may be a precursor to SFP (McAdie and Keeling, 2002; Rodenburg *et al*., 2003; Van Krimpen, 2012). It is important that research focuses on elucidating whether GFP may act as a precursor to SFP and associated plumage damage (Rodenburg *et al*., 2013).

Feather-pecking has been identified as one of the most significant welfare concerns in laying hens due to its high frequency of occurrence and damaging nature (Bestman *et al*., 2009; Gilani *et al*., 2013). Severe FP also causes a negative economic impact due to increased energy demands of denuded birds and higher mortalities due to cannibalism (Tullett *et al*., 1980; Gunnarsson *et al*., 1999; Yamak and Sarica, 2012; Rodenburg *et al*., 2013). A number of causative factors have been identified in contributing to its expression and effective strategies to address the problem are yet to be developed (Bestman and Wagenaar, 2003; Rodenburg *et al*., 2004; Lambton *et al*., 2010; Wysocki *et al*., 2010; Lambton *et al*., 2013).

One particular causative factor that has been found to be positively correlated with FP in a number of studies is the inhibition of foraging behaviours such as ground-pecking or a lack of environmental stimuli (Blokhuis and Arkes, 1984; Huber-Eicher and Wechsler, 1997; Dixon *et al*., 2010; Gilani *et al*., 2013). It has been suggested that the inhibition of environmental pecking or foraging may cause the redirection of would-be environmental pecks towards the feathers of conspecifics (Blokhuis and Arkes, 1984; Blokhuis, 1986; Huber-Eicher and Wechsler, 1998). Additionally, the rearing period and early life experiences are thought to be important for the development of FP behaviours later in life (Blokhuis and Van Der Haar, 1992; Johnsen *et al*., 1998).

Many studies have investigated the effect of providing environmental enrichment (EE) to encourage foraging and to alleviate the incidence of SFP (Blokhuis and Van Der Haar, 1992; Huber-Eicher and Wechsler, 1997; Jones, 2001; Jones *et al*., 2002). However, the optimal type of enrichment to decrease the incidence of SFP has yet to be determined (Huber-Eicher and Wechsler, 1997; Sambrook and Buchanan-Smith, 1997). Furthermore, studies have found equivocal results when investigating the relationship between EE, the expression of foraging behaviours in the rearing period and SFP in the laying period (Johnsen *et al*., 1998; Newberry *et al*., 2007; De Jong *et al*., 2013; Rodenburg *et al*., 2013). Hence, the optimum type and quantity of enrichment as well as the time period and duration in which it is provided requires further investigation.

Due to its multifactorial nature, SFP is not well-understood and is largely controlled by remedial measures such as beak-trimming (BT) and reduced lighting (Petek and Mckinstry, 2010), which aim to curb the problem but do not address the primary cause of the behaviour (Gilani *et al*., 2013). Additionally, BT is a contentious issue that presents welfare concerns of its own, including acute and chronic pain (Gentle, 1986; Petek and Mckinstry, 2010). Countries such as Switzerland, Sweden, Norway, and Finland have banned BT, with other countries including the UK proposing future bans (van Horne and Achterbosch, 2008; Petek and Mckinstry, 2010; Gilani *et al*., 2013; Rodenburg *et al*., 2013). Hence, there is a need for studies to investigate the expression of SFP with and without the use of BT.

The main objectives of this experiment were 1) to investigate whether BT and EE during the rearing period affects plumage damage in the laying period and 2) to investigate the relationship between behaviour in the rearing period and plumage damage in the laying period. EE was provided in the form of whole oats, pecking strings, and extra litter to stimulate foraging and other exploratory behaviours. BT was performed at 1 d with a follow up light trim at 11 weeks of age. Birds are often re-trimmed between 8 and 12 weeks of age in Australia in order to prevent re-growth of the beak tip and subsequent damage due to SFP (Glatz *et al*., 2009). A key objective of the present experiment was to investigate the effect of the removal of the tip of the beak. Due to subsequent regrowth by 11 weeks of age, a second light trim was deemed necessary to address a key objective of the experiment.

MATERIALS AND METHODS

Animals, housing and husbandry

All experimental procedures in this study were conducted in accordance with the University of Sydney Animal Ethics Committee approved protocol and with the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2004). Eight-hundred and fifty ISA Brown chicks were obtained from a commercial hatchery at 1 d of age and placed into 16 pens measuring 1.83 m × 3.25 m. This number was reduced to 800 by 16 weeks of age to leave a total of 50 birds per pen for the remainder of the experiment. This gave a stocking density of approximately 8.4 birds per m^2 in each pen and 2.1 birds per m^2 including the range area. Water was provided in bell drinkers and feed in scratch trays for the first 10 d then in feed troughs thereafter. Feed and water were available *ad libitum*. Chicks were fed a commercial crumbled pullet starter feed for the first five weeks, a commercial pullet grower feed to 18 weeks, and commercial coarse crumble layer feed thereafter (Vella Stock Feeds, Sydney, Australia). Each pen contained a timber perch unit comprising five parallel perches (125 cm by 4 cm) at five different heights from week 13, and a 10-hole nest box unit from week 15 with two rows of five single-bird nests and perches at the front of each row (Figure 1).

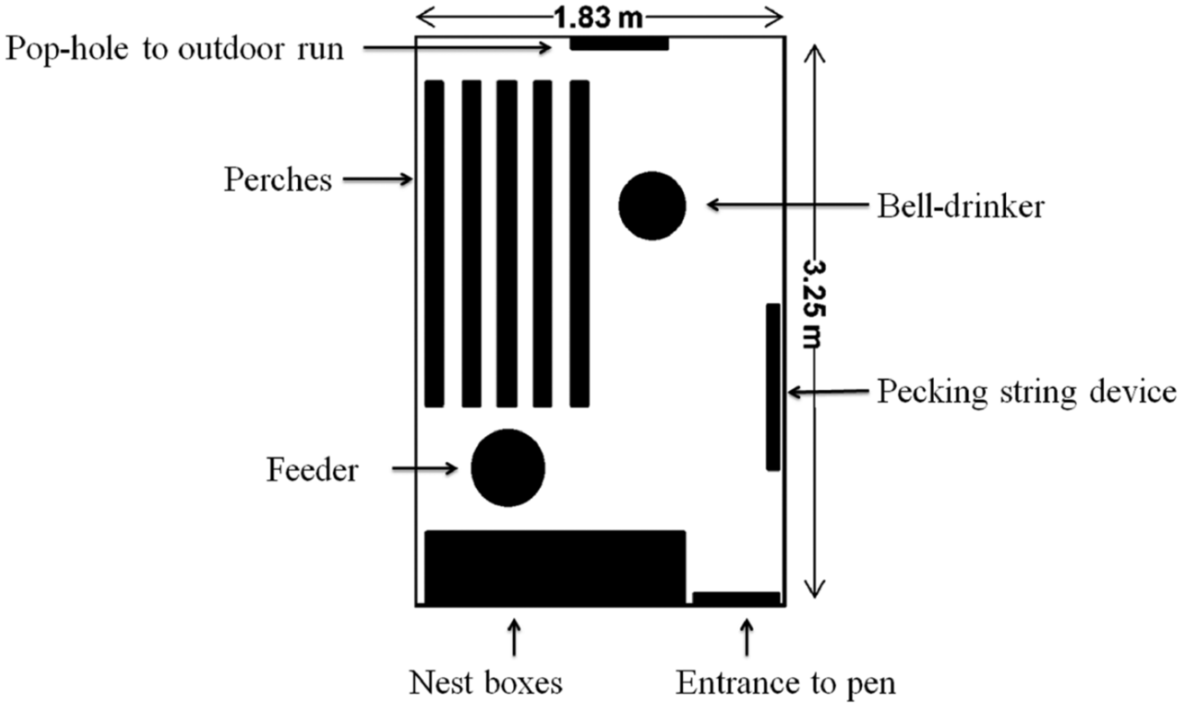

Figure 1. Illustration of pen layout (without range area)

Birds were housed in a naturally ventilated shed with wood shavings spread over a solid concrete floor and reared in accordance with recommended conditions (ISA Brown Commercial Management Guide, 2010). Heat lamps (250 Watt, Caldo Bello Brooder Heaters, Sydney, Australia) were provided in each pen to maintain the temperature at approximately 34°C for the first 7 d. The temperature was progressively reduced by 2°C per week until ambient temperature was reached and the heat lamps were removed at five weeks of age. Artificial lighting was provided via fluorescent tubes with some natural light entering the shed during daylight hours.

Artificial lighting was provided for 12 h per day to 13 weeks of age. Between 14 and 19 week the lighting schedule was increased by 30 min per day until a photoperiod of 15L:9D was reached. Light levels within the shed were measured using a Tonda j Digital Lux Meter (Model number: LX-1010B), (Instrument Choice, Laboratory Equipment Supplier, Dry Creek, SA). Nine readings were taken in each pen at the front, centre, and rear of the pen at ground level, 1 m from the floor and 2 m from the floor. The readings were taken eight times, on two sunny and two overcast days prior to the pop-holes being opened and on two sunny and two overcast days after the pop-holes were opened. Light measurements were averaged across all pens, giving 52 lux for the shed. All pens were given continuous access to outdoor runs from 26 weeks of age. At this age at least 80% hen-day egg production had been recorded for all pens. Outdoor runs were 1.83 m wide × 10 m long and consisted of 2.1 m high wire mesh fences and wire mesh across the top of all pens. Each run contained a small winter garden area measuring approximately 2.4 m in length comprising 1.2 m of metal-roofed verandah and 1.2 m shade cloth material. The open range area was initially covered with grass and then dirt once grass had been denuded by birds and contained no other vegetation or artificial structures.

Treatments

EE and BT were applied in a 2 × 2 factorial arrangement and blocked according to the side of the shed (north and south). Treatments were randomized to pens within blocks. Half of the birds were laser-BT at 1 d of age at the hatchery using a Novatech infra-red laser unit and received a follow-up light-trim with a hot blade on-farm at 11 weeks of age. The latter procedure was performed by a certified professional BT contractor where approximately one fifth of the top and bottom mandibles were removed (the tip of the beak). Beaks were cut and cauterized with the hot blade and subsequently dipped in ice water to ensure any residual heat from the hot blade was dissipated rapidly. Following this, a topical analgesic (Tri-Solfen, Bayer Australia Ltd, Wyong NSW) was then immediately applied to the trimmed beaks as was recommended by the University of Sydney Animal Ethics Committee. The other half of birds was not BT and thus had entirely intact beaks throughout the experiment.

Enrichment was provided from 12 d of age in three forms, the first was the provision of five pecking string devices per pen. Pecking strings were based on the design described by Jones *et al*. (2002) and McAdie *et al*. (2005) and comprised bundles of white polypropylene baling twine that were suspended at approximate bird head height. The second form of enrichment was the inclusion of whole oats to encourage foraging and food-searching behaviour in the litter. Approximately 150 g of whole oats were strewn over the litter three to five times weekly in each enriched pen. The final form of enrichment was the provision of deeper litter. Enriched pens received additional wood shavings to give an approximate litter depth of 50 mm maintained throughout the rearing period. Pens with no enrichment did not have pecking strings or whole oats provided and had a litter depth of approximately 10 mm.

Focal birds

Four focal birds from each of the 16 pens were selected at random using a random number generator at 11 d of age for behaviour observations. The 64 focal birds were wing-tagged and feathers on their backs and wings were spray-marked with non-toxic colored paint for identification purposes (dark blue, light blue, green, and pink). They were then subjected to detailed in situ behaviour observations from 3 weeks of age.

In situ behaviour observations

Observations of focal birds' behaviour in the home pens were conducted using a similar method to those described in McAdie and Keeling (2002) and Dixon and Duncan (2010). Observers stood quietly in front of the pens in order to view bird behaviour through the wire on the front of each pen. Bird behaviour was deemed to be unaffected by observers' presence as the birds were habituated to daily human presence in the shed. Each focal bird was observed for a 2-min period that was classified as one observation session. Forty observation sessions were conducted for each bird over the rearing period from three to 14 weeks of age. Each 2-min observation session was divided into 30 s intervals in which behaviours were recorded in a binomial fashion where they were either "present" or "absent". Birds and pens were all observed in a random order each time (balancing for blocks).

Behaviours recorded during observations were those thought to be potentially related to FP (Blokhuis, 1986; Vestergaard *et al*., 1993) and were based on definitions listed in the behaviour catalogue in Nicol *et al*. (2009). These included dust-bathing, ground-scratching, beak-wiping, receiving gentle pecks, receiving severe pecks, and pecking behaviours directed at any surface. Pecking behaviours included ground-pecking, pecking vertical surfaces, pecking strings in enriched pens, GFP, and SFP. Incidents in which a bird engaged in a confrontation or had an aggressive encounter with another bird were also recorded. GFP was recorded when a peck did not appear to involve the grasping or pulling of feathers and was not administered with force.

SFP was administered with force and was recorded if a peck involved the grasping or pulling of feathers or both. It sometimes resulted in the removal and subsequent ingestion of feathers and at times appeared to cause discomfort or pain when the receiver vocalised and retreated or performed a retaliatory peck.

Production measurements

Feed intake and egg production were measured weekly on a per pen basis. Eggs were collected daily, counted four times weekly, and weighed once each week. Feed conversion ratios were calculated using grams of feed consumed, divided by the average egg mass for each pen per day. Egg production as well as whether eggs were laid on the floor, in nest boxes, or in the outside range areas were recorded from 17 to 35 weeks of age. Birds were individually weighed, feather-scored and examined for injuries every four to six weeks throughout the experiment from 11 d to 43 weeks of age. As plumage damage appeared to be cumulative, only the feather-scores from week 43 were used in analysis.

Feather-scoring

Plumage damage has been found to be strongly correlated with SFP in previous studies (Bilčík and Keeling, 1999). Experimenters in the present study were confident that the plumage damage observed was due to SFP rather than other factors, as birds were all individually examined at four to six week intervals throughout the experiment. In addition, feather loss patterns (on the rump, tail, and back regions) coupled with wounds and missing flesh appeared dissimilar to feather loss associated with molting. There was also no feather loss due to cage abrasions as they were housed in a non-cage system. Anecdotal observations of the birds as well as observations as part of a later study supported the notion that plumage damage was due to SFP. Hence, detailed feather-scoring was conducted for each focal bird individually as an indication of SFP in each pen. The feather-scoring system was adapted from the method described by Tauson *et al*. (2005). Each bird was assigned a score between 0 and 4 for each body area representing integument condition, feather-coverage, and plumage damage (Table 1). The body areas that were scored were the head, neck, back, sides, belly, vent, tail, and rump. A diagram of the body areas can be seen in Bilčík and Keeling (1999). The same observers carried out feather-scoring each time to ensure consistency in scoring procedure.

Table 1. Feather-scoring system adapted from Tauson *et al*. (2005)

Feather-score	Description
0	No damage, perfect feather condition
1	Feather damage visible, but no significant bare patches of note
2	Some damage with a small patch or patches of bare skin lacking feather-coverage. Size of bare patch dependent on body area – minority of feathers on body area removed (usually a few centimeters in diameter)
3	Extensive feather loss and bare patches with medium to large areas that had been denuded. Majority of body area denuded (dimensions of bare patches dependent on body area)
4	Completely denuded areas or with bloody or wounded areas or both. In severe cases, flesh had been pecked out where feather-pecking had escalated into cannibalism

Statistical analysis

Behaviour observations

Data were analysed using the logistic Generalized Linear Mixed Models procedure with a binomial distribution in GenStat (15^{th} edition). For all behaviours, the fixed effects model was the two treatment factors (BT, EE) and their interaction, with the pen and block as the random effects model. Interaction was dropped and fixed effects were analysed independently where there was no interaction effect ($P > 0.05$) on a behaviour. The effect of behavioural measures (as proportions of total time budget) on feather-scores was then analysed using ordinal regression using the ordinal library in R (version 3.0.1). Fixed effects were the behavioural measures, and pen and block were the random effects. This analysis was done to assess the relationship between behaviour in the rearing period and SFP in the laying period in isolation from treatment effects.

Feather-scores

The feather-scores for each bird were analysed using ordinal regression in ASReml 3 using the highest (worst) score out of all body parts scored for each bird at 43 weeks of age. Fixed effects were BT, EE, and their interactions, with pen and block as random effects.

Production data

Production data were averaged for all weeks (17 to 42) prior to analysis and the experimental unit for all production measurements was the pen of birds. Treatment effects on production measurements including feed conversion ratio, hen-day production, feed intake, cumulative egg production, body weight, and proportion of floor eggs within each pen were analysed using the

Linear Mixed Models procedure in the GenStat (15th edition). The effect of range access on production measurements was analysed by compiling data for production weeks prior to opening the pop-holes and for the weeks after the pop-holes were opened to the cessation of the experiment. Data were then analysed using the Linear Mixed Models procedure. Data were checked for normality, and log-transformed (if required).

RESULTS

In situ behaviour observations

Birds that had been BT performed less ground-pecking (P = 0.003, 45% vs 34%) and SFP (P = 0.02, 0.41% vs. 0.02%) but more GFP (P = 0.02, 2.1% vs. 3%) and also tended to perform more beak-wiping (P = 0.08, 1.9% vs. 2.8%) during observations than non-trimmed birds. Birds in EE pens were observed to perform more ground-scratching (P = 0.03, 13% vs. 9%) than those without EE, and there was an interaction effect on dust-bathing where non-trimmed birds with no EE were observed to perform less dustbathing (P = 0.01). There was also a trend for an interaction effect on pecking vertical objects where trimmed birds with EE performed the fewest vertical pecks (P = 0.08).There was no effect of treatment on social confrontations (P = 0.33), receiving gentle (P = 0.42) or severe (P = 0.99) feather-pecks, and no effect of BT on pecking at strings (P = 1.0) in enriched pens.

When behaviours were analysed as predictor variables separately from the effects of BT and EE to correct for treatment effects, there was a positive association (P = 0.03) between ground-pecking during rearing and feather-score in week 43. There was also a trend where birds performing more beak-wiping when young tended to have better feather condition when older (*P* = 0.09). All other behaviours were not associated with feather-scores in week 43 (all P > 0.10).

Feather-scoring

When feather-scored in week 43, BT birds had better plumage condition than those with intact beaks (P < 0.001, Figure 2), denoted by lower scores. While some pens of birds exhibited no plumage damage, some of the non-trimmed pens of birds had high feather scores with large areas of missing feathers, wounds and some missing flesh. The body areas primarily affected were the back, rump, and tail. There was no effect of EE (*P* = 0.27) and no interaction effect (*P* = 0.81).

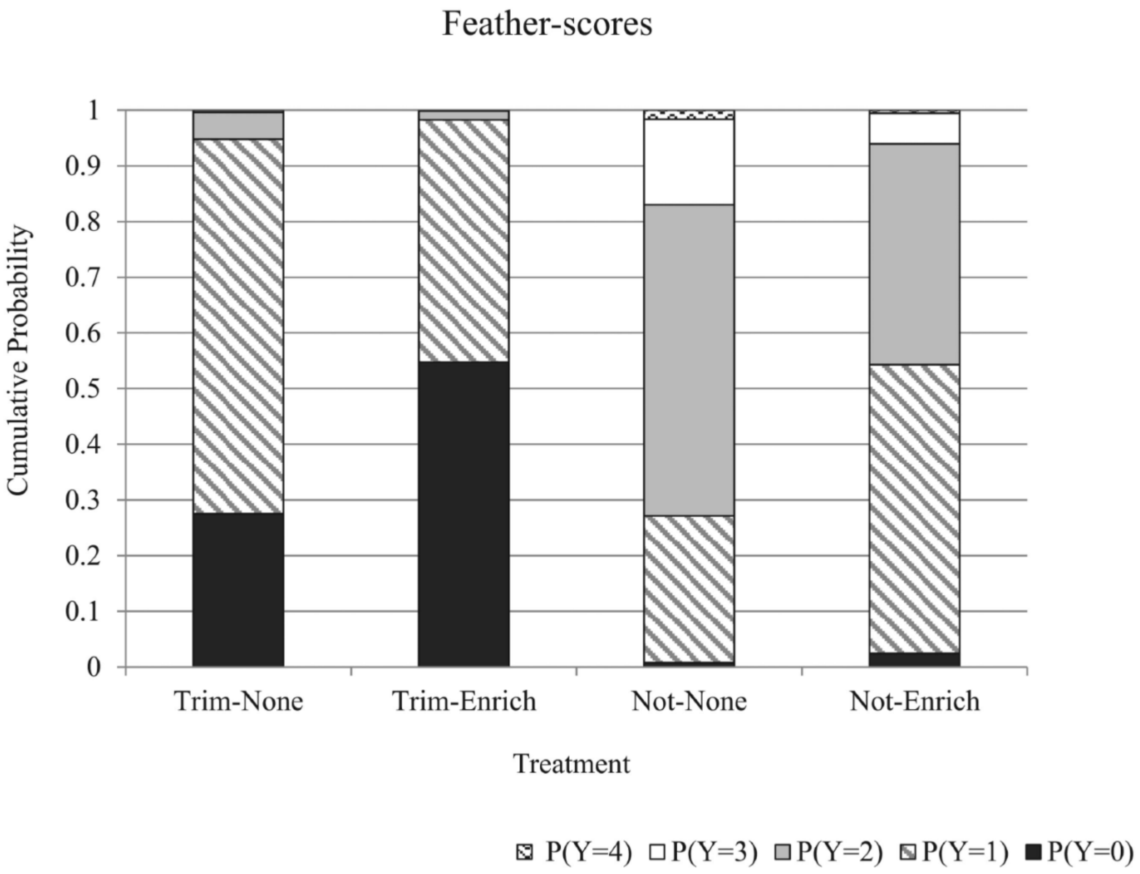

Figure 2. Model-based probability distribution of feather-scores of focal birds at 43 weeks of age as a measurement of severe feather-pecking.

Production Data

There were no effects of treatment on feed conversion ratio ($P = 0.23$), feed intake ($P = 0.47$), live body weight ($P = 0.85$), cumulative egg production ($P = 0.31$), hen-day production ($P = 0.31$), or proportion of floor eggs ($P = 0.15$) throughout the experiment. There was an effect of range access on the proportion of floor eggs, where fewer eggs were laid on the floor and more in the lower nest boxes (P < 0.001, 48% vs. 32%) after the pop-holes were opened.

DISCUSSION

Environmental enrichment

EE during the rearing period had no effect on plumage damage in the laying period. This finding contrast with some other studies including Blokhuis and Van Der Haar (1989) and Johnsen *et al*. (1998) where the provision of EE during the rearing period was found to decrease FP in the laying period. Blokhuis and Van Der Haar (1992) found that additional grain supplied during rearing in floor pens with litter floors resulted in a decrease in feather damage due to FP in the laying period. They theorised that this may be due to the enhanced "incentive value" of the ground due to the provision of additional grain. Similarly, Nørgaard-Nielsen *et al*. (1993) and Nørgaard-Nielsen and Lawson (1995) found that FP was more common in barren environments than in environments with high incentive value, and that EE decreased the incidence of plumage deterioration due to FP. Despite contrasting with some previous studies, the results from the current experiment were congruent with findings by De Jong *et al*. (2013) where there was no effect of early life experience on behaviour later in life. Dixon and Duncan (2010) also found that the provision of substrate for foraging during the first 2 weeks of age did not affect FP in week 3 and 4.

An important aspect to consider when interpreting the results is the type of enrichment used and the time period in which it was provided. Van Krimpen (2012) and Johnsen *et al*. (1998) reported that experience in the first four weeks of life has a large influence on the development of FP. The treatments in this experiment were only applied from day 12, hence, skipping the first two weeks of what may be an important developmental period. Blokhuis and Van Der Haar (1992) stated that not only the "peckability" of the ground is important, but other sensory factors such as taste and nutritive value are important as well. Huber-Eicher and Wechsler (1998) also found that the quality as well as the quantity and availability of foraging materials had an effect on foraging behaviour and feather-pecking. The hens in this experiment may not have been interested in the type of grain supplied, as was observed anecdotally, and supported by the lack of an effect of enrichment on the incidence of ground-pecking observed during direct observations. Studies have found that litter floors decrease the incidence of FP when compared to floors without litter (Blokhuis and Arkes, 1984; Blokhuis and Van Der Haar, 1989). Additionally, De Haas *et al*. (2014) found that disruption and limitation of litter supply early in life increased plumage damage and SFP. Hence, if birds without EE had been housed on slats rather than litter in this experiment, it may have acted to heighten differentiation between treatments.

Beak-trimming

Non-trimmed birds showed more plumage damage than BT birds at 43 weeks of age. Bolhuis *et al*. (2009) and Lambton *et al*. (2010) also found that plumage damage was lower in BT birds. As the non-trimmed birds performed more SFP in the rearing period in the current experiment, the higher levels of plumage damage in week 43 may be attributed to heightened frequencies of SFP in these birds in the laying period (Huber-Eicher and Sebo, 2001). However, SFP was measured by plumage damage and not by direct observations in week 43. Hence, it is possible that all birds performed SFP at the same frequencies during the laying period but that the non-trimmed birds achieved greater effectiveness due to their intact beaks. Indeed, Blokhuis and Van Der Haar (1989) found that despite non-trimmed groups of birds exhibiting higher levels of feather damage, BT did not affect the frequency of FP. BT may, therefore, act to reduce plumage damage due to SFP rather than affect the frequency of occurrence.

Lambton *et al*. (2010) found that birds that had been BT in the rearing period had the highest rates of GFP during rearing on commercial farms and also had reduced plumage damage later in life. It was suggested that although SFP presents the greatest economic and welfare concern, GFP may also have a negative impact on bird welfare. Gentle FP may manifest as a stereotypic behaviour where birds' needs are not being met (Rodenburg *et al*., 2013). It has been theorised that BT may inhibit general exploratory behaviour such as ground-pecking (Blokhuis and Van Der Haar, 1989), and that GFP may develop as a stereotypic response to this (Lambton *et al*., 2010). Freire *et al*. (2011) explained that minor BT causes reduced mechanoreception and magnetoreception in the beak. It was observed that BT chicks performed what were described as compensatory pecks to account for the lower sensory feedback. In that experiment, trimmed birds pecked harder at a pecking stimulus than non-trimmed birds in the first 24 h after trimming. The higher incidence of GFP observed in trimmed birds in the present experiment may be explained by the impaired functionality of the magnetoreceptors and mechanoreceptors in the beak. If GFP is a socially explorative behaviour, as described by Riedstra and Groothuis (2002), birds with trimmed beaks may increase the incidence of this behaviour, giving it a compensatory nature. The phenomenon of impaired sensory feedback from trimmed beaks and the consequent increased rate of pecking has also been reported in other studies (Gentle *et al*., 1997). The higher rate of beak-wiping in the BT birds in the present experiment may also be attributed to compromised sensory feedback experienced by these birds, leading to compensatory beak-wipes.

Birds with intact beaks performed more SFP and more foraging in the form of ground-pecking in the rearing period than BT birds. These birds had poorer feather-scores in the laying period. Birds that perform more ground-pecking and SFP when young may perform more SFP and exhibit poorer plumage condition when older due to higher activity levels (Bilčík and Keeling, 2000; Huber-Eicher and Sebo, 2001; Newberry *et al*., 2007). The findings from the present experiment are in agreement with Newberry *et al*. (2007) who found a positive relationship between foraging in rearing and SFP later in life (between 17 and 37 weeks of age) and also with the results in Bilčík and Keeling (2000), who found a positive relationship between ground-pecking and SFP on an individual bird level between 22 and 37 weeks of age. However, foraging behaviours may not be an indication of the propensity to peck due to the inhibitive nature that BT imposes on the expression of certain pecking behaviours. BT birds most likely experience decreased sensitivity in the form of reduced magnetoreception and mechanoreception (Freire *et* *al*., 2011), which could hinder them from performing pecking behaviours directed at the ground. As found in Blokhuis and Van Der Haar (1989), there was no difference in the frequency of SFP between birds that had been BT and those with intact beaks despite worse plumage condition in birds with intact beaks. This could imply that BT birds have the same motivation to perform SFP but are unable to achieve the same pecking efficiency to cause plumage damage as birds with intact beaks (Blokhuis and Van Der Haar, 1989). Hence, the positive association between ground-pecking in the rearing period and plumage damage in the laying period could merely be an expression of the behavioural opportunities of BT verses non-trimmed birds.

Behaviour in rearing

There were some effects of EE on behaviours during the rearing period but no effect on plumage damage in week 43. This is consistent with findings by De Jong *et al*. (2013) who found bird behaviour to adapt due to environmental conditions, but that these effects may not be evident later in life when enrichment is no longer present.

Birds with intact beaks performed less GFP and more SFP during rearing than birds that had been BT. This may indicate that GFP is not linked with SFP and may have a different underlying motivation such as exploration (Newberry *et al*., 2007). In the laying period, the non-trimmed birds exhibited more plumage damage. Hence, there was no association between the incidence of GFP when young and plumage damage when older. It could also be theorised that SFP in the rearing period may predict plumage damage later in life. Huber-Eicher and Sebo (2001) also found FP to increase in occurrence over time and that future work should focus on the development of FP in the rearing period.

Effects on production

There were no effects of BT and EE on production. However, the provision of range access affected the site of egg laying and acted to reduce the proportion of floor eggs. Opening the pop-holes to provide range access caused some birds to relocate their laying site, with more eggs recorded in the nest boxes rather than on the floor. Some hens therefore altered their chosen laying sites. Hence, environmental factors applied during the laying period may act to influence nesting site selection and affect proportion of eggs laid in nest boxes compared with other locations such as the floor. This information may be of practical significance as floor eggs require manual collection and can be downgraded, hence, causing a negative economic impact (Cronin *et al*., 2013).

CONCLUSIONS

This experiment found a negative association between BT in rearing, and plumage damage at 43 weeks of age but no effect of EE. Focal birds that had not been BT performed more ground-pecking and SFP but less GFP during the rearing period. These birds then exhibited more plumage damage later in life. The results indicate that BT performed in the rearing period may be an effective method to reduce the expression of SFP in the laying period.

ACKNOWLEDGMENTS

The Australian Egg Corporation provided funding for this experiment. The Poultry Cooperative Research Centre, established and supported under the Australian Government’s Cooperative Research Centres Program, provided a postgraduate stipend for the first author. The advice of

Dr. Phil Glatz (South Australian Research and Development Institute) on beak-trimming is acknowledged.

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CHAPTER 4: **Plumage damage in free-range laying hens**: **Behavioural characteristics in the rearing period and the effects of environmental enrichment and beak-trimming**

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Published in: *Applied Animal Behaviour Science* (2015)

164:64-72

OVERVIEW OF CHAPTER 4

The review in chapter 2 identified that more information is needed on behaviour during the rearing period, and how it relates to severe feather-pecking in the laying period. The introduction in chapter 1 also outlined the need for information on the underlying behavioural characteristics of birds, and how this relates to their propensity to perform severe feather-pecking. Research is required to investigate the effects of environmental enrichment during rearing on bird behaviour, in terms of fearfulness and sociality. Studies have found that severe feather-pecking may be selected against. Information is required to identify behavioural characteristics of birds which may be predisposed to perform severe feather-pecking, in order to develop standardised phenotypic selection traits. The experiment in chapter 4 was therefore conducted to investigate the effects of environmental enrichment on bird behaviour in behavioural tests during the rearing period. In addition, the predictive value of behavioural tests during rearing for severe feather-pecking later in life was also assessed. This experiment investigated the effects of beak-trimming and environmental enrichment during rearing on pullet responses to behavioural tests, and how this relates to the development of severe feather-pecking in free-range laying hens.

ABSTRACT

Severe feather-pecking, whereby birds peck at and pull out the feathers of other birds, is one of the greatest welfare concerns and the most prevalent behavioural problem in laying hens. It can be extremely difficult to control, especially in non-cage laying flocks. Despite a multitude of studies on the topic, the principal underlying causes remain unclear and not much is known about why certain birds are affected more than others. Literature suggests that rearing is an important period for the development of behaviours later in life. Although severe feather-pecking is not usually a welfare concern in the rearing period, behavioural tests when performed early in life may be predictive of plumage damage due to severe feather-pecking in adulthood. This experiment aimed to investigate whether behavioural tests during the rearing period could be predictive of plumage damage later in life. Sixteen pens of 50 ISA Brown laying hens were used, with four birds per pen selected at random as focal birds. Focal birds were subjected to behavioural tests during the rearing period including the open-field test, tonic immobility test and tests for a novel food reward. Two treatments, beak-trimming and environmental enrichment, were applied in a 2 × 2 factorial arrangement in rearing. The non-trimmed birds vocalised more ($P = 0.02$, 91.5 vs. 83.6%) and at louder volumes ($P = 0.02$, 71.4 vs. 47.0% of vocalisations categorised as loud rather than soft or silent) in the open-field test at five weeks of age. There was no difference between treatments in duration of tonic immobility ($P = 0.99$). Non-trimmed birds exhibited more plumage damage at 43 weeks of age ($P < 0.001$, 5.2 vs. 72.9% of birds with feather loss or wounds). Ordinal regression with treatments and treatment interactions as fixed effects and pens and blocks (sides of the shed) as random effects indicated no significant associations between behavioural test reactions and plumage damage (all $P > 0.1$). Thus while beak trimmed birds made fewer vocalisations in an open-field test and had less plumage damage in adulthood as expected, there is no evidence that reactions to the behavioural tests were predictive of plumage damage. Instead, results indicate that EE affected bird behaviour during the rearing period but did not affect plumage damage due to severe feather-pecking later in life. The test responses including more vocalisations in the open-field test, but no difference in the tonic immobility responses, indicate that the differences may be due to motivation for social reinstatement rather than fearfulness.

Keywords: feather-pecking, free-range, laying hen, plumage damage, open-field test, tonic immobility

INTRODUCTION

Severe feather-pecking (SFP) is a behaviour performed by birds which usually involves the vigorous pecking at or pulling of feathers of conspecifics. It can lead to extensive feather loss, acute injuries and sometimes death (Savory, 1995, McAdie and Keeling, 2000). It is regarded as a highly prevalent detrimental behaviour which presents a significant welfare problem in laying hens (*Gallus gallus domesticus*) (Gunnarsson *et al*., 1999, Bestman *et al*., 2009, Gilani *et al*., 2013). Severe feather-pecking can be a more serious problem in non-cage housing systems where birds are typically housed in larger group sizes and it is therefore more difficult to control (McAdie and Keeling, 2002, McAdie *et al*., 2005, Gilani *et al*., 2013).

It is widely acknowledged that laying fowl engage in social encounters that include aggressive pecking, usually targeted towards the head and neck regions, in order to establish and maintain hierarchical structures within groups. However, SFP is identified as being distinct from aggressive pecking, with different underlying behavioural motivations and etiologies (Savory, 1995). It is recognised as a persistent multifactorial behaviour with a wide variety of causative factors including environmental, social and genetic. Despite a multitude of studies, the underlying behavioural causes of SFP are unknown and effective strategies to address the problem are yet to be developed (Bilčík and Keeling, 2000, Lambton *et al*., 2013). It has been suggested that the rearing period and early life experiences are thought to be important for the development of SFP later in life (Blokhuis and van der Haar, 1992, Johnsen *et al*., 1998). Although many studies have investigated the effect of environmental enrichment (EE) on SFP and plumage damage, they have yielded equivocal findings (Johnsen *et al*., 1998, De Jong *et al*.,

2013) and the optimal type of enrichment has yet to be determined (Huber-Eicher and Wechsler, 1997, Sambrook and Buchanan-Smith, 1997).

Due to the lack of information on the causes of SFP and no current effective method of prevention, SFP is widely controlled by remedial measures such as beak-trimming (BT) which presents a contentious issue and is banned or due to be banned in a number of countries in Europe (Petek and McKinstry, 2010, Gilani *et al.*, 2013). Hence there is a need for studies to investigate the expression of SFP with and without the use of BT as well as a need for alternatives to be researched such as effective EE as a means to prevent SFP from developing. Enrichment is considered to be of particular importance during the rearing period (Blokhuis and van der Haar, 1992, Johnsen *et al.*, 1998) and acts to both increase the behavioural repertoire of birds and reduce fearfulness and SFP (Jones and Waddington, 1992, Huber-Eicher and Wechsler, 1997, Jones *et al.*, 2002). Jones *et al.* (2002) found bunches of white string to be a practicable and effective form of enrichment in order to divert injurious pecking away from other birds. McAdie *et al.* (2005) also found similar string devices to decrease feather pecking and improve feather condition. Similarly, foraging materials are thought to affect the expression of feather pecking and plumage damage, particularly when provided in the rearing period (Rodenburg *et al.*, 2013).

Previous studies have indicated that behaviour during the rearing period such as activity levels in an open-field test (OFT) may be predictive of the development of SFP later in life (Rodenburg *et al.*, 2004). Behavioural tests such as those involving novel food items, the OFT and the tonic immobility (TI) test, when performed in the rearing period, may provide useful information relating to underlying behavioural traits as well as the developmental causes of SFP (de Haas *et al*., 2010, Rodenburg *et al*., 2003, 2004). The TI test and OFT have been widely used and are well validated tests in poultry (Forkman *et al*., 2007). As SFP is thought to be related to foraging behaviour as well as fearfulness and sociality (Jones *et al*., 1995, de Haas *et al*., 2010), tests performed early in life involving novel food items, social proximity and removal of conspecifics may yield additional information on the underlying behaviour and predictive characteristics of birds that may be more prone to developing damage due to SFP.

Although there is thought to be an association between fearfulness and SFP, the causal relationship is not known (Jones *et al*., 1995). By investigating levels of fearfulness in the rearing period and plumage damage due to SFP in the laying period, this could yield information about the relationship and whether fearful birds may be predisposed to plumage damage. Different behavioural characteristics between birds could potentially affect their propensity to receive SFP. Birds with lower levels of fearfulness may be more or less prone to plumage damage due to SFP because of behavioural responses when SFP is aimed at them. For example, less fearful birds may be more likely to retaliate to SFP and therefore less likely to be targeted for SFP in the future. Ascertaining behavioural characteristics of birds in the rearing period and comparing these with plumage damage in the laying period could provide information on the role fearfulness in the development of plumage damage due to SFP.

The objectives of the current experiment were (1) to investigate the effect of BT and EE (applied in a 2 × 2 factorial design) on behaviour in the rearing period and (2) to evaluate the usefulness of behavioural tests performed in the rearing period and their relationship with plumage damage later in life. EE was provided from 12 days of age in the form of whole oats distributed in the litter, extra litter depth and pecking strings comprising white polypropylene baling twine. BT was performed at 1 day of age with an infrared laser unit and a follow-up light-trim with a hot blade at 11 weeks of age. EE and BT were both predicted to reduce plumage damage in the laying period. EE was expected to reduce fearfulness in behavioural tests during rearing whereas BT may act to enhance it. Birds assessed as more fearful during the rearing period were predicted to exhibit more plumage damage in the laying period.

MATERIALS AND METHODS

Animals, housing and husbandry

Eight-hundred and fifty ISA Brown chicks were obtained from a commercial hatchery at 1 day of age and placed into 16 pens measuring 1.83 m × 3.25 m. The total number of birds was reduced to 800 by 16 weeks of age to give 50 birds per pen for the laying period. Water and a commercial crumbled feed were provided ad libitum via one red bell drinker (T-40 Bell Drinker, Tecnica e Innovaciones Ganaderas, S.A., Spain) and one feeder (25 kg Jumbo Feed Hopper, Protective Fabrications, Werombi, NSW, Australia) per pen. Scratch trays for feed were provided for the first 10 days. Birds were housed in a naturally ventilated shed with wood shavings spread over a solid concrete floor. Artificial lighting was provided for 12 h per day to 13 weeks of age via fluorescent tubes with some natural light entering the shed during daylight hours. The photoperiod was adjusted by 30 min per week from 14 weeks to provide 15 h light and 9 h dark at 19 weeks of age. Outdoor range areas measuring 1.83 m × 10 m per pen were continuously accessible via a pop-hole in each pen from 26 weeks of age. Each pen contained a five-rung timber perch unit from 13 weeks of age. Each of the five perches was 4 cm wide and 125 cm long. A 10-hole nest box unit was provided from 15 weeks of age (SKA 10 Hole Rollaway Nest, Bellsouth Poultry Equipment eshop, Narre Warren, VIC, Australia).

All experimental procedures were conducted in accordance with the University of Sydney Animal Ethics Committee approved protocol and with the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2004). Birds were reared in accordance with recommended conditions (ISA Brown Commercial Management Guide, 2010).

Focal birds

A total of 64 birds (four per pen) were selected as focal birds using a random number generator when live weights were first recorded, at 11 days of age. These birds were fitted with wing-tags and leg rings. Feathers on the backs and wings were also spray-marked with non-toxic coloured paint for identification purposes. Spray-marking was conducted approximately every 2 weeks and all focal birds were spray-marked on the same day each time.

Treatments

BT and EE were applied in the rearing in period in a 2 × 2 factorial arrangement and blocked according to side of the shed (north and south). BT was performed at the hatchery at 1 day of age when the tip of the beak was heat-treated (Nova-tech infra-red laser unit, Nova-Tech Engineering, MN, USA). A follow-up light-trim was performed at 11 weeks of age with a hot blade, when approximately one-fifth of the top and bottom mandibles removed (i.e. the tip of the beak). EE was provided from 12 days of age in the form of five white polypropylene pecking string devices per pen (Jones *et al*., 2002, McAdie *et al*., 2005), average litter depth of approximately 50 mm and 150 g of whole oats distributed throughout the pens three to five times weekly. Pens with no enrichment did not have pecking strings or whole oats and litter depth was maintained at approximately 10 mm throughout the rearing period. Differentiated litter depth between treatments and provision of whole oats ceased at weeks 15 and 20, respectively.

Tonic immobility test

The TI test was performed on each focal bird individually as a measure of fearfulness at 9 weeks of age using a similar method to those described by Jones and Faure (1981) and Campo *et al*. (2008). Order of pens and birds for all testing procedures was randomised but balanced for fixed and random effects. Once selected for testing, each focal bird was removed from their home pen individually and carried in an upright position to the area of the shed designated for behavioural testing. Tonic immobility was induced immediately after the bird was placed onto a table. The table sloped gently to the centre where there was a slight depression. A thin layer of wood shavings was placed on the table and separated in the centre to accentuate the depression and attempt to mimic the function of the U-shaped cradle that has been utilised in previous studies (Jones and Faure, 1981). This also acted to minimise birds inadvertently rolling from the TI position onto their sides, which may interrupt the TI catatonic response. Each bird was gently turned onto its back and restrained by two experimenters applying firm but gentle pressure onto the breast and legs whilst simultaneously covering the head and eyes for a period of 10 s. Once

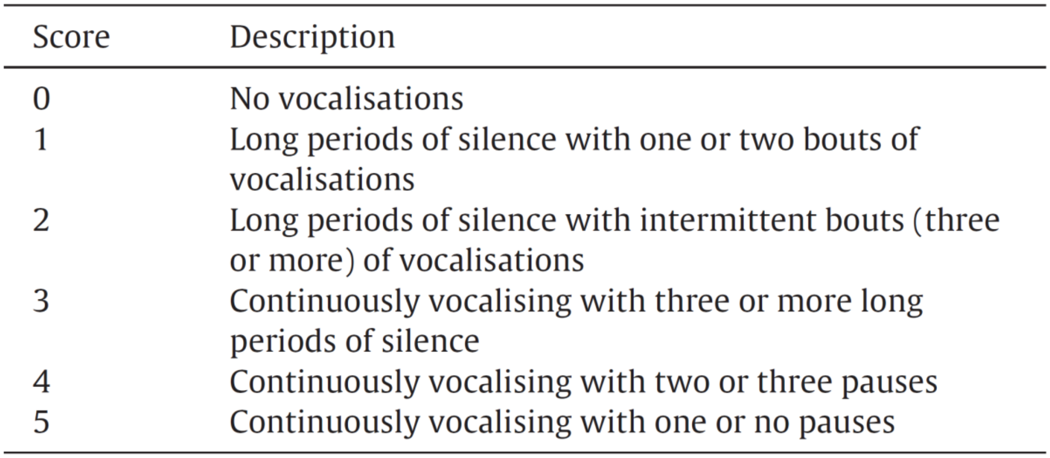
TI had been induced, observers' hands were removed and latency until self-righting was recorded by the use of a stopwatch while observers stood motionless and quietly in view of the bird. Self-righting was considered as having occurred when the bird had resumed a standing posture on both feet. If the TI reaction did not persist for a minimum duration of 14 s, the induction procedure was repeated until the bird maintained TI for at least 14 s. If this was not achievable after five attempts the bird was returned to the home pen and tested at a later time. Tonic immobility was terminated at 180 s. If the bird maintained TI until 180 s, the test was stopped and the bird was manually righted by an observer and assigned the maximum score of 180 s. The test was performed at one end of the shed out of view of other birds. Hence birds were unaffected by the visual presence of other birds but may have been affected by auditory or olfactory cues within the shed that were unnoticed by experimenters. The same two experimenters conducted all behavioural testing.

Open-field test

Each focal bird was subjected to a 5-min OFT at 5 weeks and again at 13 weeks of age using similar methods to Buitenhuis *et al*. (2004) and Rodenburg *et al*. (2003). Similar to the TI test, the OFT was performed inside the shed out of view of other birds. Birds were individually placed in a 1.3 m × 1.3 m test arena with 0.8 m high white-painted walls and a linoleum floor. A 3 × 3 grid was marked on the floor with black lines to facilitate the quantification of bird activity levels. Birds were placed in the same starting position in the arena (Figure 1) by the same observers for consistency. A transparent lid comprised of chicken wire was slowly lowered over the top of the arena via a pulley system following bird placement. The following variables were then recorded: quantity and volume of vocalisations, latency to initial step, latency to exit the starting square, number of grid lines that were crossed, number of defecations and the number of escape attempts or attempts to fly. Continuous data were recorded every 30 s and then collated to provide a total for each 5-min period for analysis.

Two video cameras with sound were positioned 3 m above the test arena, 1 m apart. Bird movements were observed on a video monitor located away from the test arena to avoid human interference with test responses. Activity levels were quantified by using the 3 × 3 grid marked on the floor and counting the number of grid crossings made by each bird in the 5 min time period (Figure 1). The quantity of vocalisations made by each bird was recorded using an ascending scale from 0 (no vocalisations) to 5 (continuously vocalising with one or no pauses) (Table 1). The volume of vocalisations was recorded by the use of a subjectively defined 3-tiered scale, allocating the vocalisations made in each 30 s interval a score of ‘silent’, ‘soft’ or ‘loud’.

Table 1. Scoring system for quantity of vocalisations over a 30 s time period. Bout = Three or more vocalisations in a row. Pause = 2 s. Long period of silence $\geq$ 3 s.



Score	Description
0	No vocalisations
1	Long periods of silence with one or two bouts of vocalisations
2	Long periods of silence with intermittent bouts (three or more) of vocalisations
3	Continuously vocalising with three or more long periods of silence
4	Continuously vocalising with two or three pauses
5	Continuously vocalising with one or no pauses

Habituation to test arena for further testing

To minimise the effect of fear of the test arena interfering with bird responses during subsequent behavioural tests, focal birds were provided with an opportunity to familiarise themselves with the test arena. This procedure was referred to as ‘habituation’ and was performed in pairs of focal birds from the same pen at 9 weeks of age. Birds were placed in the arena in pairs due to the fearful and distracted responses that they exhibited when observers attempted to habituate them individually. When in the presence of a conspecific, birds appeared less frightened and more interested in a food reward.

Two birds were removed from the same home pen and placed into the test arena simultaneously (Figure 1). A meal worm (*Tenebrio molitor*) was then placed on the floor in front of each bird concurrently and observers waited until both birds ingested one meal worm each before removing the birds from the arena. The procedure was performed once per bird. Observers noted that when birds were introduced to the test arena without prior habituation they were not responsive to a food reward. Following habituation, birds were able to be tested.

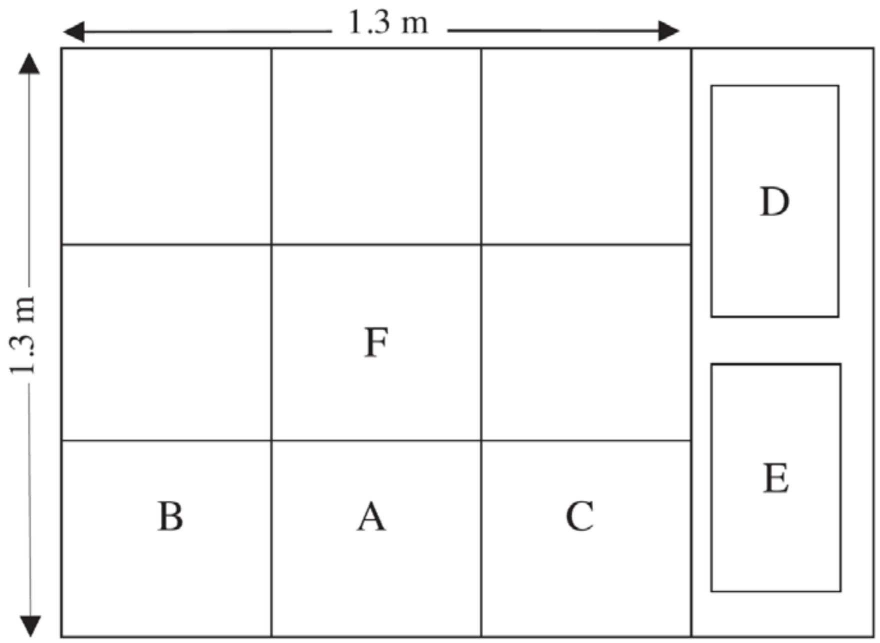


Figure 1. Placement of focal birds in test arena. (A) Starting position of birds for open-field test. (B) and (C) Starting positions of two focal birds during habituation. (D) and (E) Position of birds following placement for competition test and individual test. (F) Placement of meal worm for individual and competition tests.

Individual response to a food reward in the test arena

Immediately following habituation, birds were individually placed in the test arena to record their latency to obtain a novel food reward. Focal birds were placed in a starting box (Figure 1) on the side of the test arena and a stopwatch was started. After 10 s had elapsed, a meal worm was placed in the centre of the arena by one observer and another observer watched the video monitor. The worm was dropped into the arena thereby allowing the experimenter to remain out of sight. The bird was able to see the placement of the worm but unable to access it due to a transparent door comprised of chicken wire. After another 10 s the door separating the bird from the arena was opened by the use of a pulley so as to not distract the bird by the presence of observers. After the door was raised, each bird was given a maximum of 30 s to ingest the meal worm. The bird was removed from the arena or starting box when it had consumed the meal worm or when 30 s had elapsed. The maximum latency of 30 s was assigned to birds that did not ingest the meal worm. The test was conducted twice per bird, once from a starting box on the left of the arena, and once from a starting box on the right. Meal worms were used in this test as they are known to be an attractive food for chickens and this was readily observed in the present experiment. The order of pens and birds for testing was random, balanced for fixed and random effects in order to minimise potential confounding effects such as prior feed ingestion. Test reactions were observed via the video monitor so that experimenters did not influence birds' responses.

Competition test

The conduct of the competition test was of a similar design as the individual test. After being habituated to the test arena and then tested individually, each focal bird was then tested in the company of a conspecific focal bird from the same pen. Both birds were placed simultaneously in the starting boxes by two observers, one bird in the left starting box and the other in the right. One meal worm was dropped into the centre of the arena in sight of the birds after 10 s had elapsed. The barrier separating the birds from the arena was raised 10 s later. The latency of one of the birds to obtain the meal worm was recorded. The four focal birds from each pen were tested in pairs, so the test was conducted six times per pen for all potential pairing combinations. The two birds waiting to be tested were placed in a 'holding pen' and at the completion of the tests all four focal birds were returned to their home pen. Birds were not kept in the holding pen individually and were always in the company of at least one other pen mate.

Feather-scoring

The amount of SFP received has been found to be strongly related with plumage damage (Bilčík and Keeling, 1999). Hence detailed feather-scoring was conducted for each focal bird at 43 weeks of age to assess plumage damage and hence indicate the extent of SFP in each pen. When plumage damage occurred in a pen it was widespread and affected the majority of birds. Birds with extensive plumage damage were also observed to administer SFP. The method used for feather-scoring was adapted from Tauson *et al*. (2005). Each bird was assigned an ordinal score for each body area representing feather condition, feather-coverage and wounds. Scores ranged from no damage (0) through to completely denuded, bloody or wounded areas (4). Body areas scored were head, neck, back, sides, belly, vent, tail and rump. A diagram of the body areas can be seen in Bilčík and Keeling (1999). The same observers conducted feather-scoring each time to ensure consistency in scoring procedure.

Statistical analysis

To test the association between behavioural responses in the rearing period and plumage damage later in life, data were analysed using an ordinal generalised linear mixed model in R (version 3.0.1) with the ‘ordinal’ package. Bird responses in behavioural tests were used as predictor variables of plumage damage in week 43. For each bird the worst score (out of scores from all body parts) was used for analysis as the outcome variable with pens and blocks as the random effects.

Open-field test and competition test

Data for the number of grid crossings, escape attempts and defecations in the OFT were analysed using the generalised linear mixed models procedure with Poisson distribution in GenStat 15th edition. Linear mixed models were used to analyse latency to obtain the food reward in the competition test and latencies in the OFT. As each bird was tested multiple times in the competition test, the average latency for each bird to ‘win’ the food reward was used. Missing values where neither bird acquired the food reward were excluded from analysis, and data were log-transformed prior to analysis if not normally distributed.

BT and EE (and their interaction) were the fixed effects with pen and block as the random effects in both models.

Open-field test vocalisations and feather-scores

An ordinal logistic generalised linear mixed model was fitted to the ordinal vocalisation (6-point scale) and volume (3-point scale) data using ASReml 3.0 (VSN International, Hemel Hempstead, UK). The predictor variables were calculated from the OFT data as follows: The number of times each ordinal category was allocated for each bird was multiplied by the score for each category, and then summed to create a measure for quantity of vocalisations. Volume was calculated similarly as a sum of the weighted scores where Silent = 1, Soft = 2 and Loud = 3.

BT, EE and their interaction were fixed effects with pens and blocks as the random effects. Due to the nature of an ordinal scoring system, feather-scores for each bird were analysed using the ‘worst score’ from all body parts for each bird.

Tonic immobility and individual test

As the TI and individual test reactions were terminated at 180 s and 30 s, respectively, and included random effects (pen and block), data were analysed using Cox's proportional hazards model using the 'survival' package in R (version 3.0.1), with BT and EE and their interaction as the fixed effects.

RESULTS

Open-field test

In the OFT in week 5, birds from enriched environments took longer to perform their initial step than those from non-enriched pens (P = 0.02, 6.17 s vs. 2.31 s). There was no treatment effect on latency for birds to move out of the starting square in which they were placed (P = 0.23), and no effect on the incidence of escape attempts (P = 0.93) or number of defecations (P = 0.45). There was an interaction effect on activity levels where BT birds from enriched pens performed fewer movements (P = 0.02, Figure 2). Non-trimmed birds vocalised more (P = 0.02, Figure 3) and at louder volumes (P = 0.02, Figure 4) than BT birds.

When the OFT was performed at 13 weeks of age there were no effects of treatment on any behaviours including latency to initial step (P = 0.65), time to cross initial boundary (P = 0.65), activity levels (P = 0.40) or quantity (P = 0.21) and volume (P = 0.12) of vocalisations.

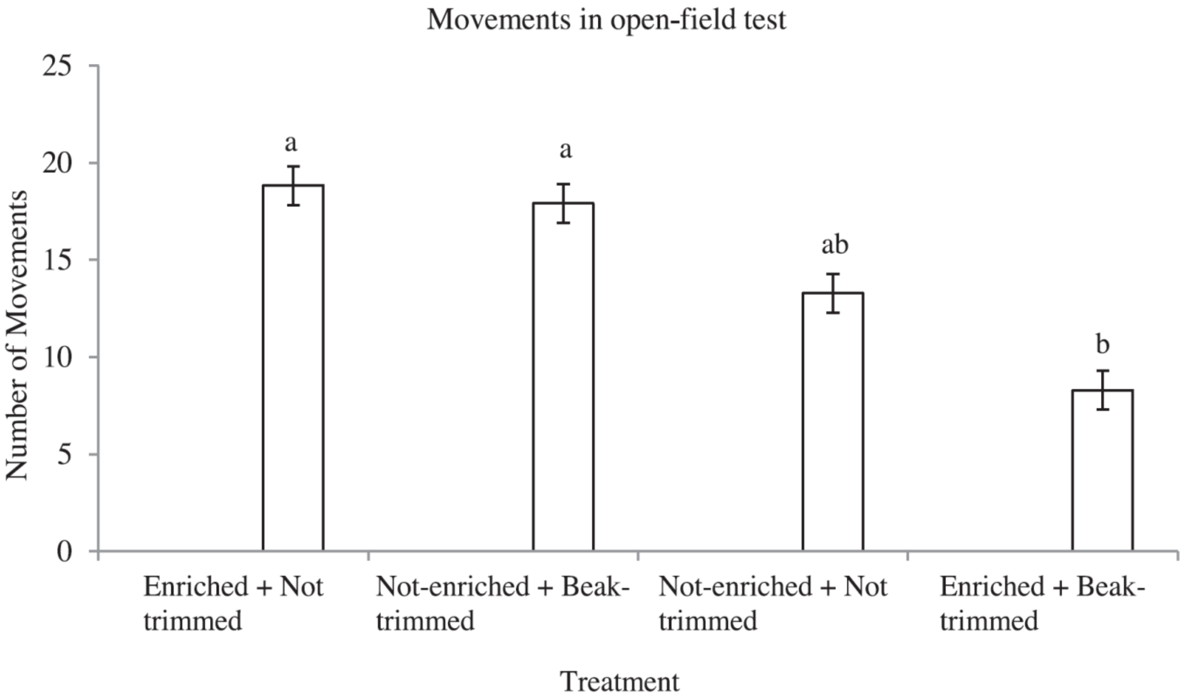


Figure 2. Activity levels in the open-field test in week 5, where enriched pens received greater litter depth, pecking strings and whole oats in the litter. Columns with different superscripts differ significantly ($P < 0.05$).

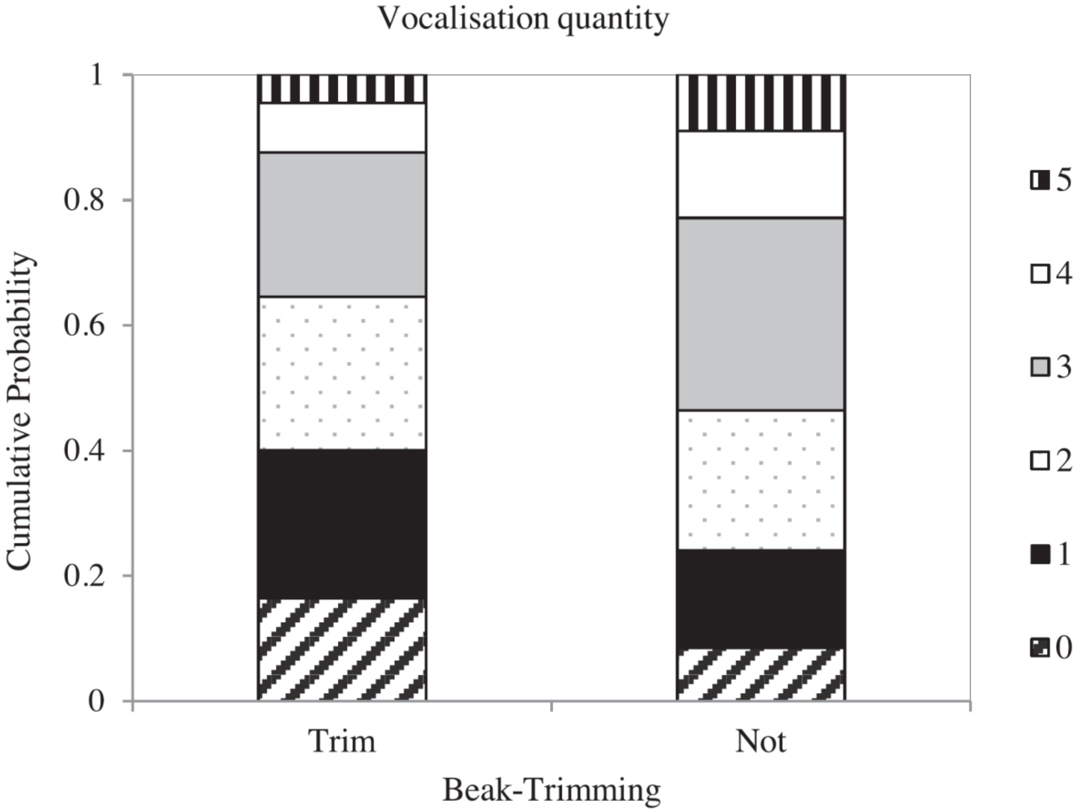


Figure. 3. Score distributions shown as cumulative probabilities of vocalisations by focal birds in the open-field test in week 5 on an ascending scale of 0–5.

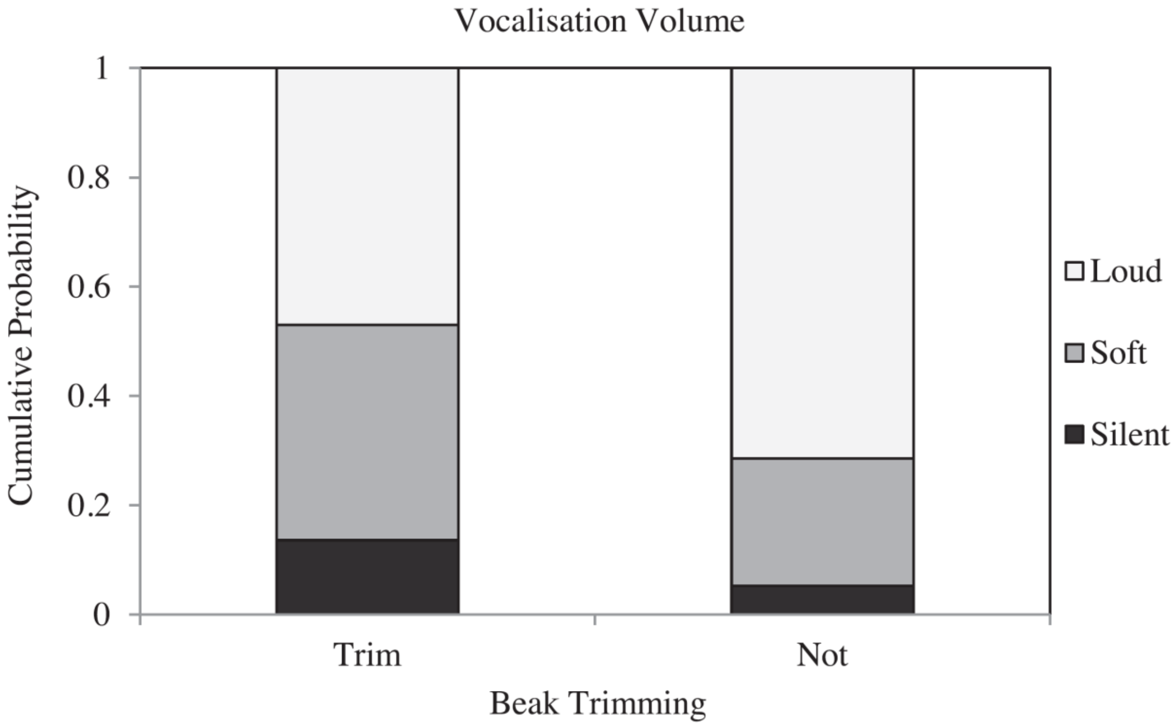


Figure 4. Volume of vocalisations by focal birds in the open-field test in week 5.

Tonic immobility and individual test

There was no interaction effect (P = 0.99) and no effect of EE (P = 0.79) or BT (P = 0.96) on TI test response. Similarly, there was no interaction effect (P = 0.15), no effect of enrichment (P = 0.75) and no effect of BT (P = 0.87) on individual test response.

Competition test

There was an interaction effect of treatment (P = 0.04) when birds were competing with another bird for a food reward. Birds that had been BT and were also from enriched pens had longer latencies to ‘win’ a food reward than birds from other treatment combinations when in the presence of another bird (Figure 5).

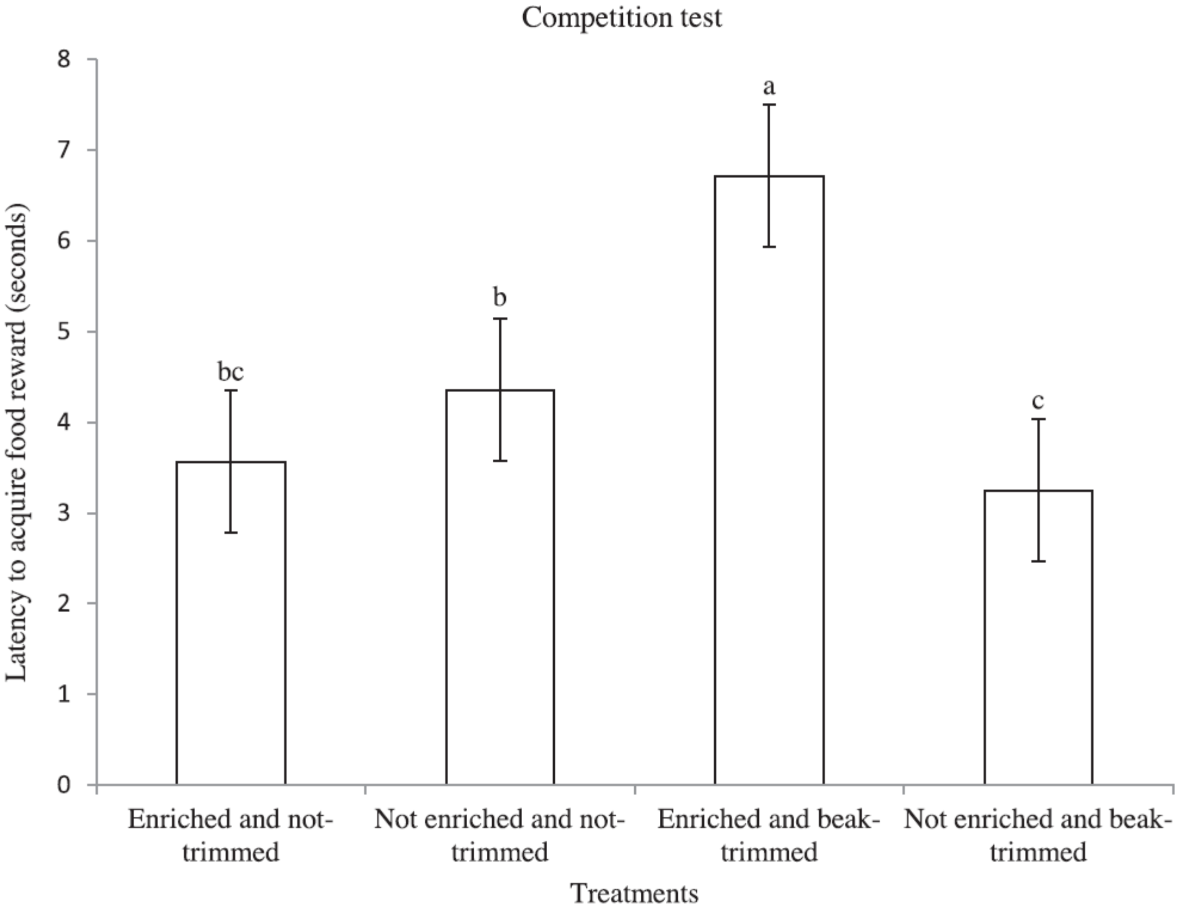


Figure 5. Latency to acquire a novel food reward in the presence of another focal bird, where birds from enriched pens received greater litter depth, pecking strings and whole oats in the litter. Columns with different superscripts differ significantly ($P < 0.05$).

Plumage damage

Birds with intact beaks exhibited more plumage damage than trimmed birds in week 43 ($P < 0.001$, average worst score 2.71 vs. 1.66). There was no effect of enrichment ($P = 0.27$). When test reactions in week 5 were compared with plumage damage in week 43 by means of ordinal regression, there were no significant associations (all $P > 0.1$).

DISCUSSION

Effect of treatments

Differentiated litter depth between treatments and the provision of whole oats was only applied in the rearing period and ceased at 15 and 20 weeks of age, respectively. There was no subsequent effect on plumage damage in week 43. This contrasts with some previous studies (Blokhuis and van der Haar, 1989, Johnsen *et al.*, 1998). The lack of an effect on plumage damage in the present study may be due to the type of enrichment as well as the time period in which it was provided. Johnsen *et al*. (1998) found that the first 4 weeks of life have a major effect on feather pecking later in life. Enrichment in the present experiment was only provided from 12 days of age. Nevertheless, it appeared that enrichment had an effect on bird behaviour whilst present, as also reported by De Jong *et al*. (2013). The effects of EE on behaviour during rearing could be indicative of the underlying behaviour of birds in enriched environments rather than providing predictive information about the development of SFP later in life.

Tonic immobility

There were no significant differences in TI latencies between birds that exhibited plumage damage in the laying period and those that did not. There were also no treatment effects on TI responses. Similarly, Jones *et al*. (1995) found no difference in TI durations between high and low feather-pecking lines. It was suggested in Jones *et al*. (1995) that predator evasion behaviour may have overcome birds' motivation for social reinstatement. The lack of difference found in the present study may be due to high levels of fearfulness across treatment groups. High levels of fearfulness may predominate over other opposing tendencies such as motivation to stand upright and social reinstatement (Jones *et al*., 1995).

Heightened fearfulness in the present experiment may be attributed to the age at which the test was performed, with younger birds sometimes exhibiting heightened fearfulness and motivation for social reinstatement when compared to older counterparts (Rodenburg *et al*., 2003). However, other studies have used the TI test early in life and found differentiated responses (Jones and Faure, 1981, Riedstra and Groothuis, 2002). A heightened fear response in the present experiment may have been due to environmental conditions such as location of the test, number of observers, noises, etc. Alternatively, bird responses in the TI test may have been affected by the regularity of handling. Regular handling is thought to have an effect on the duration of TI response, where birds that are regularly handled are thought to have shorter TI durations (Jones and Faure, 1981). Birds in the present study were handled regularly due to a number of reasons including individual weighing and feather-scoring, spray-marking for identification, changing leg rings as the birds grew, etc. Hence the TI responses may have been affected due to the regular handling that the focal birds experienced.

Open-field test

Birds from enriched pens took longer to perform their initial step in the OFT at 5 weeks of age. BT birds from enriched pens performed fewer movements in the OFT in week 5 and also had longer latencies to ingest a novel food reward when tested in the presence of a conspecific. BT birds vocalised less and at quieter volumes than birds with intact beaks.

Bird responses in the OFT are thought to be related to fearfulness and motivation for social reinstatement, reflecting a compromise between opposing tendencies for social reinstatement and avoiding detection by predators (Suarez and Gallup, 1983, Jones *et al*., 1995). Studies suggest that locomotion and vocalisations in the OFT appear to be influenced by fear, where a less active response suggests a more fearful bird (Suarez and Gallup, 1983). Decreased fearfulness is typically indicated by higher activity levels in novel environments such as the OFT and tests involving novel food items, and shorter latencies in TI tests (Jones *et al*., 1995, de Haas *et al*., 2010).

Bolhuis *et al*. (2009) found that BT reduced fearfulness, where a more active response in a manual restraint test was interpreted as a reduction in fearfulness. There was also less plumage damage in BT birds in week 40. It was suggested that although the birds in that study were less fearful than non-trimmed birds, that undergoing the procedure of BT early in life would be expected to enhance fearfulness in chicks (Bolhuis *et al*., 2009). In the present experiment, the trimmed birds made fewer vocalisations and at lower volumes than non-trimmed birds. The trimmed birds may have therefore exhibited more fearful responses.

Fewer vocalisations in an OFT may indicate a more fearful response. However, vocalisations in the current experiment in week 5, which were identified as distress calls (Rodenburg and Koene, 2003), may alternatively represent a higher level of fearfulness. Indeed, both active and passive coping styles may indicate fear (Bolhuis *et al.*, 2009). Hence another interpretation is that the acute stress of BT may have advanced the rate of development of some behavioural and physiological processes leading to less fear in fear-provoking settings. The early literature on rodents show that early handling results in reduced emotionality and defecation in an OFT as well as more appropriate endocrine responses to adult stress (Dewsbury, 1992).

In addition to fearfulness, OFT reactions provide information relating to motivation for social reinstatement (Suarez and Gallup, 1983). Higher levels of ambulation and more vocalisations in the OFT are thought to be indicative of higher social motivation (Suarez and Gallup, 1983, Jones *et al.*, 1995, Forkman *et al.*, 2007). In the present experiment, the birds which had undergone BT performed fewer movements and made fewer vocalisations. However, there were no differences between responses in the TI test. The duration of TI is a relatively straightforward, well-validated method of estimating fearfulness in birds. Conversely, although the OFT is also widely used by ethologists in estimating fearfulness, it can be more complicated to interpret test reactions. This is because birds’ reactions in the OFT may be viewed as either proactive or reactive responses to fearful stimuli or the motivation for social reinstatement. That is, a more active response in the OFT may be interpreted as either a heightened motivation for social reinstatement or a proactive response to fear-provoking stimuli (Forkman *et al.*, 2007). In the present experiment, although there were differing reactions between treatments in the OFT, there was no difference when birds were exposed to the TI test. This may indicate that the birds' responses to the OFT stemmed from social motivation rather than differing levels of fearfulness.

Ages

The same four focal birds from each pen were tested in the OFT in weeks 5 and 13. Hence the lack of a difference between treatments in week 13 may have been due to habituation to the test arena and desensitisation to social isolation. Alternatively, the older age at which the birds were re-tested may have contributed to a lack of responses. Rodenburg *et al*. (2003) suggested that fearfulness and social motivation to return to the flock is not as strong in adult laying hens compared with chicks.

Competition test

Latencies to ingest a novel food reward when birds were placed into the test arena individually did not differ between treatments. However, when birds were tested in the presence of a conspecific, there was an interaction effect on latency where BT birds from enriched environments took longer to acquire the food reward. The same pens of birds also exhibited lower activity levels in the OFT in week 5. Jones *et al*. (1995) interpreted ambulation in the OFT as a sign of higher social motivation and also that birds with higher social motivation may have the ability to interact more successfully with other birds. Hence the BT birds from enriched pens in the present experiment may have compromised success in relation to interactions with pen mates.

Relationship with plumage damage

There were some treatment effects in the behavioural tests in week 5, but no association between behavioural test reactions and plumage damage. Although there was no relationship between TI test latencies and plumage damage, there was a difference between pens in the amount of plumage damage. This suggests that there was no relationship between fearfulness in the rearing period and plumage damage in the laying period. Previous studies have reported higher levels of fearfulness in birds affected by SFP (Hughes and Duncan, 1972, Rodenburg *et al*., 2013). Results from the present experiment suggest that higher fearfulness may be caused by SFP and the associated plumage damage rather than fearfulness acting as a predictor of SFP. This contrasts with other studies which have found predictive relationships between fearfulness during rearing and SFP later in life (Rodenburg *et al*., 2004). There were differences between treatments in responses to tests associated with sociality, but no association with plumage damage. Hence it appears that there was no relationship between social motivation and victimisation in this study.

CONCLUSION

The reactions of focal birds in behavioural tests conducted during the rearing period were not predictive of plumage damage at 43 weeks of age. There were some treatment differences apparent in the OFT at 5 weeks of age which may indicate a difference in social motivation. BT birds made fewer vocalisations and at lower volumes in the OFT at 5 weeks of age and had not developed plumage damage by 43 weeks of age. This suggests that BT may cause a decreased need for social reinstatement at a young age and also decrease plumage damage later in life.

Although the current study did not find a relationship between behavioural tests in the rearing period and plumage damage later in life, studies of this nature are important in order to contribute to the current understanding of the underlying behavioural causes of SFP. Future experiments should investigate fearfulness at the time that SFP is being performed, potentially focusing on investigating the behaviour of birds performing SFP compared with those which are victimised. This could provide further information and an enhanced understanding about the underlying behavioural motivations behind SFP.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Australian Egg Corporation Limited for providing funding for the experiment and the Australian Poultry Cooperative Research Centre for providing a post-graduate stipend for the first author.

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CHAPTER 5: Underlying behavioural characteristics of free-range laying hens involved in severe feather-pecking – ‘peckers’ versus ‘victims’

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OVERVIEW OF CHAPTER 5

The importance of understanding the underlying behavioural differences between birds was highlighted in chapters 1, 2 and 4. In particular, fearfulness and social motivation may be particularly important traits which are involved in the expression of severe feather-pecking. Unexpectedly, we did not find any relationships between behaviour in the tests during rearing, and subsequent plumage damage in the laying period in chapter 4. Further characterisation of the birds performing SFP was therefore required. As outlined in chapters 1 and 2, individual bird variation is a particularly important avenue of research. Investigating severe feather-pecking behaviour between individual birds and associated behavioural traits may have the potential to contribute to genetic selection programs. The experiment in chapter 5 was therefore conducted to obtain information about birds in the life phase in which they were performing severe feather-pecking. Birds were categorised based on their involvement in severe feather-pecking, as either recipients of severe feather-pecking, or the performers of severe feather-pecking; ‘peckers’ or ‘victims’. Categorising birds according to their role in severe feather-pecking has the potential to improve our understanding of whether certain behavioural characteristics may be associated with the expression of severe feather-pecking, whether performing or receiving it.

ABSTRACT

Severe feather-pecking is the most prevalent behavioural problem in the egg industry worldwide. It is a multifactorial behaviour which can lead to extensive feather loss, wounds, and possibly cannibalism. Severe feather-pecking therefore represents a serious risk to animal welfare. Individual bird differences are an important source of information when investigating the underlying behavioural causes, and coping style in response to fearful stimuli may be associated with the propensity to develop severe feather-pecking. Environmental enrichment has been investigated as a potential means to abate fearfulness in birds, encouraging foraging behaviours, and preventing or alleviating severe feather-pecking behaviour. The present study investigated the behavioural characteristics of birds which perform severe feather-pecking, ‘peckers’, compared with recipients of severe feather-pecking, ‘victims’, and control birds. Comparisons were also made at a pen level, between pens with extensive plumage damage, and those with little plumage damage. Half of the pens had been provided with environmental enrichment during rearing (between 12 days and 20 weeks of age), which included pecking strings, deeper litter, and whole oats distributed in the litter. Focal birds were subjected to an open-field test and a tonic immobility test between 35 and 42 weeks of age, and behaviour in the home pen was observed between 43 and 44 weeks of age. In the open-field test, peckers had greater quantities ($P < 0.001$) and higher volumes ($P < 0.001$) of vocalisations than victims, and also made more escape attempts ($P = 0.03$). In the tonic immobility test, victims had longer latencies to right themselves than peckers ($P < 0.001$). While there was no effect of enrichment on plumage damage ($P = 0.44$), birds from pens which had received enrichment tended to receive fewer severe feather-pecks during observations (P = 0.06). Enrichment was provided from 12 days to 20 weeks of age. Thereby birds did not have enrichment at an early age, nor at the time of testing, which may have compromised its effectiveness. Longer latencies in the tonic immobility test suggest that birds with the most extensive plumage damage also had higher levels of fearfulness. More vocalisations and escape attempts in the open-field test, and shorter latencies in the tonic immobility test, suggest that peckers may be more proactive in response to fear-provoking situations, which is in agreement with previous literature. The higher levels of vocalisations and escape attempts made by peckers may have been due to greater social motivation.

Key words: severe feather-pecking, behaviour, tonic immobility, open-field test, free-range, fearfulness

INTRODUCTION

Severe feather-pecking (SFP) is a particularly injurious behaviour whereby birds vigorously peck at, and pull out the feathers of conspecifics. It can lead to extensive feather loss and wounds, and sometimes cannibalistic pecking (Rodenburg *et al*., 2013). SFP poses significant welfare and economic problems in the egg industry worldwide, and in particular, in non-cage production systems (McAdie and Keeling, 2002; Rodenburg *et al.*, 2010; Gilani *et al*., 2013). Despite numerous studies, and the identification of multiple causal factors, effective methods for the prevention and control of SFP are sought (Lambton *et al*., 2013).

Kjaer *et al*. (2015) presented evidence to suggest that it is not a stereotypic behaviour. However, SFP appears to have persistent and compulsive characteristics, similar to trichotillomania in humans and barbering in mice, and may have similar underlying causes (Garner *et al*., 2004; Kops *et al*., 2013). Environmental enrichment (EE) has been trialled as a means to mitigate SFP (Huber-Eicher and Wechsler, 1997; Jones *et al*., 2002), and is thought to enhance the behavioural repertoire and decrease fearfulness (Jones and Waddington, 1992). Enrichment is particularly influential when provided during the rearing period (Blokhuis and van der Haar, 1992; Johnsen *et al*., 1998). Importantly, while there are strong environmental influences, it is known that certain individual birds in a flock can express SFP while others do not (Rodenburg *et al*., 2004a; Jensen *et al*., 2005). This can occur despite identical housing environments, diets, and donor flocks. Hence, there appears to be individual bird variation within groups of birds that cannot be accounted for by investigating external factors (Jensen *et al*., 2005; Brunberg *et al*., 2011; Kops *et al*., 2013). This variation should be taken into account when investigating the causes of SFP (Rodenburg *et al*., 2013).

SFP is thought to be associated with fearfulness (Hughes and Duncan, 1972; Jones *et al*., 1995; Rodenburg *et al*., 2004b), and differences in SFP between individuals may be a reflection of different coping strategies in response to a stressor. It has been suggested that fearful birds are more likely to develop SFP (Rodenburg *et al*., 2004b), and also that SFP causes increased fearfulness in birds which are victimised (Hughes and Duncan, 1972). While Rodenburg *et al*. (2013) identified the role of fear and stress as one of the approaches providing the most scope for a sustainable solution for SFP, the relationship between fearfulness, foraging behaviour and SFP is not well understood (de Haas *et al*., 2010), and it has yet to be established whether the 'victims' or the birds performing the severe pecks are more fearful (Jones *et al*., 1995).

It has been acknowledged that SFP should primarily be considered as 'the interaction between an individual bird and its environment' (Hughes and Duncan, 1972). However, despite an awareness of individual variation in the expression of SFP, most studies have focused on selecting lines differing in their propensity to perform SFP (Jensen *et al*., 2005). Few studies have actually focused on individuals in order to characterise the behavioural traits of birds differing in their propensity to peck, as well as the extent of their plumage damage. It has yet to be determined why birds kept under the same environmental conditions vary in their propensity to express SFP. Information relating to the behavioural characteristics of individual birds could provide insight into the development of SFP (Rodenburg *et al*., 2004a; Daigle *et al*., 2014).

Further, identifying the underlying causes of SFP is the most promising route forward when aiming to reduce the problem (Brunberg *et al*., 2011).

While it has previously been found that fearfulness and social motivation are implicated in the development of SFP (Rodenburg *et al*., 2013), in an earlier trial we found no association or predictive relationship between tests to estimate fearfulness and social motivation during rearing, and plumage damage later in life (Hartcher *et al*., 2015a). Investigating social motivation and fearfulness in the life phase in which SFP is apparent (the laying period), and comparing the behavioural traits of adult birds expressing SFP compared to those which are not, could yield important information relating to the current understanding of the expression of SFP. The present study therefore aimed to investigate the behavioural characteristics of individual birds expressing SFP, ‘peckers’, compared to those identified as ‘victims’, and ‘controls’. In addition, half of the test pens had received EE during the rearing period, in the form of pecking strings, greater litter depth and whole oats in the litter. The phenotypic associations between bird classification (peckers, victims, and controls), plumage damage, EE during rearing, behaviour in fear-inducing situations, and behaviour in the home pen were studied. Behavioural tests used were the open-field test (OFT) and tonic immobility (TI) test, which have been widely used to estimate fear responses in laying hens (Forkman *et al*., 2007). It was predicted that victimised birds would be more fearful than their SFP counterparts, and that birds provided with enrichment during rearing would express lower levels of fearfulness and SFP.

MATERIALS AND METHODS

Animals, husbandry and housing

All experimental procedures in this study were conducted in accordance with the University of Sydney Animal Ethics Committee approved protocol and with the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2004). Eight pens of 50 birds (n = 400) were used for the experiment, placed in the shed at 1 day of age. Birds were housed in an uninsulated, naturally ventilated shed in pens measuring 1.83 m × 3.25 m, each with an outside range area of 1.83 × 10 m with 2.1 m high mesh fences and wire mesh across the top. Birds were given continuous access to the outdoors via one pop-hole in each pen from 26 weeks of age. Pop-holes were 0.4 m high × 0.6 m wide, and allowed unimpeded access to the range. The range area initially had grass cover which was subsequently denuded by the birds following range access, and contained a small winter garden measuring approximately 2.4 m long. No other vegetation or artificial shade structures were provided. A commercial coarse crumble layer feed (Vella Stock Feeds, Sydney, Australia) and water were available *ad libitum.* Water was provided in red bell drinkers (T-40 Bell Drinker, Tecnica e Innovaciones Ganaderas, S.A., Spain) and feed in metal bell feeders (25 kg Jumbo Feed Hopper, Protective Fabrications, Werombi, NSW, Australia), suspended from the ceiling, which were manually re-filled. Each pen contained a timber perch unit, comprising five parallel perches (125 cm × 4 cm) at five different heights and a 10-hole nest box unit with two rows of five single-bird nests and perches in front of each row (SKA 10 Hole Rollaway Nest, Bellsouth Poultry

Equipment eshop, Narre Warren, VIC, Australia). Artificial lighting was provided by fluorescent tubes with some natural light entering the shed, giving an average light intensity of 52 lux throughout the shed. Continuous artificial light was provided for 15 hours per day during daylight hours. None of the birds were beak-trimmed.

Environmental enrichment during rearing

Half of the eight pens had been provided with EE from 12 days of age, in the form of five white polypropylene pecking string devices per pen, an average litter depth of approximately 50 mm, and 150 g of whole oats distributed on the litter three to five times weekly (Hartcher *et al*., 2015b). Pens with no enrichment did not have pecking strings or whole oats, and litter depth was maintained at approximately 10 mm throughout the rearing period. Differentiated litter depth between treatments, and the provision of whole oats ceased at weeks 15 and 20, respectively. Thereafter, all pens had approximately equal litter depth and the addition of no extra grains or additional forages. Litter depth was not decreased in non-enriched pens, but built up in both treatments, with the addition of more wood shavings. Pecking strings remained in the enriched pens for the remainder of the experiment.

Feather-scoring

Feather-scoring is highly correlated with the amount of SFP received (Bilčík and Keeling, 1999). Hence feather-scoring was conducted at 43 weeks of age for all birds (n = 400). The method used for feather-scoring was the same as described in Hartcher *et al*. (2015b), which was adapted from Tauson *et al*. (2005). Each bird was assigned an ordinal score for each body area, representing feather-coverage and wounds. Scores ranged from no damage (0) through to completely denuded, bloody or wounded areas (4). Body areas scored were the neck, back, tail and rump (the sides, vent, belly were also assessed, but no plumage damage occurred on these areas). Feather-scores were analysed using the binomial Generalised Linear Mixed Models procedure in GenStat (15th edition, VSN International, Hemel Hempstead, UK) with the number of body parts with plumage damage per bird as the outcome variable, the number of body parts scored the binomial denominator (n = 4), and the pen in the random model. The number of body parts with plumage damage was used for analyses rather than the ordinal scores due to the large quantity of zero scores in the pens without plumage damage. Half of the eight pens exhibited extensive damage, where 36.1% of body areas had plumage damage; the other four pens had very little damage, with only 3.2% of body areas with plumage damage ($P < 0.001$).

Selection of focal birds

Focal birds (n = 70) were selected for behavioural testing between 35 and 42 weeks of age. These birds were categorised as ‘peckers’ (n = 10), ‘victims’ (n = 20), or ‘controls’ (n = 40) at the time of testing. Five victims were selected from each of the plumage damaged pens as the birds with the poorest plumage condition. Five peckers were selected in two of the pens with plumage damage (one with EE, one without), via *in-situ* observations of SFP behaviour. A similar method of selection was used as in Brunberg *et al*. (2011). A bird which was seen to perform SFP was subsequently marked with coloured spray paint on the tail or wings and observed for a 10 min period. If it performed more than four severe feather pecks in the 10 min period, it was categorised as a pecker and selected for testing. Selection of peckers in the other two pens with plumage damage was not possible due to SFP occurring at lower rates. In each of the four pens with no plumage damage, 10 control birds were selected at random due to a lack of plumage damage and SFP behaviour. The victims, peckers, and controls exhibited an average of 40%, 25% and 0% of their body areas with plumage damage, respectively (P = 0.002). Categorisation of birds as ‘victims’, ‘peckers’ or ‘controls’ will be referred to as birds’ ‘classification’ for the remainder of this paper.

In order to validate the selection methods, and provide an assessment of whether the classification of peckers was reliable, *in-situ* behaviour observations were conducted between 43 and 44 weeks of age. Two pens with plumage damage and two pens with little plumage damage were observed (balanced for enrichment), using the same method as described in Hartcher *et al*. (2015b). In pens with plumage damage, three peckers and three victims were observed (n = 12). In the pens with little plumage damage, six controls were observed (n = 12) (total n = 24 birds across the four pens). Each bird was observed for a 2 min period which was classified as one observation session. Twenty-seven observation sessions were conducted for each focal bird over two weeks, giving a total of 54 mins for each bird. Each 2 min observation session was divided into 30 s intervals in which behaviours were recorded binomially as ‘present’ or ‘absent’. Birds and pens were observed in a random order, balanced for fixed and random effects. Gentle feather-pecking (GFP), SFP, aggressive pecking, receiving aggressive pecking, receiving GFP and receiving SFP were recorded. GFP was recorded when a peck did not appear to involve the grasping or pulling of feathers, was not administered with force and did not result in any damage.

In contrast, pecks were identified as severe when the bird performed a peck in a vigorous or forceful manner or grasped and pulled the feathers of another bird (Savory, 1995). Data were analysed using a binomial Generalised Linear Mixed Model in GenStat (15th edition), where the outcome variable was the number of observation periods in which a behaviour was recorded as present, and the binomial denominator was the total number of observation periods. Pen and bird were random effects, as there were repeated observations per bird, and in the fixed model, bird classification was nested within whether a pen had plumage damage or not. Peckers performed more SFP (6.9% vs 0.9% vs. 0.7% of the observed time budget for peckers, victims and controls, respectively, $P < 0.001$) and tended to perform more GFP than victims and controls (5.7% vs 2.8% vs 1.8% for peckers, victims, and controls, respectively, $P = 0.07$), but there was no effect of bird classification on SFP received ($P = 0.70$). Birds in pens with plumage damage performed more SFP (3.6% vs 0.4%, $P = 0.01$) and GFP (4.5% vs 1.4%, $P = 0.007$), and also received more SFP (5.6% vs 0.2%, $P < 0.001$).

Tonic immobility test

The TI test was performed on each focal bird individually, in the same facility in which the birds were housed. The same method was used as in Hartcher *et al*. (2015b). The order of pens and birds for testing was randomised, balanced for fixed and random effects. Once selected for testing, each focal bird was removed from their home pen individually, and carried in an upright position to the area of the shed designated for behavioural testing. TI was induced immediately after the bird was placed onto a table. Each bird was gently turned onto its back and restrained by two experimenters applying firm but gentle pressure onto the breast and legs whilst simultaneously covering the head and eyes for a period of 10 s. After 10 s, once TI had been induced, the observers' hands were removed and latency until self-righting was recorded. Observers stood motionless and quiet, in view of the bird. Eye contact was not avoided. If TI did not persist for a minimum duration of 14 s, the induction procedure was repeated until the bird maintained TI for at least 14 s. The maximum number of inductions was five. The test was terminated at 180 s.

Open-field test

The OFT was performed using the same method as described in Hartcher *et al.* (2015a). Birds were individually placed in a novel test arena for 5 min, and the following variables were recorded: quantity and volume of vocalisations, latency to initial step, latency to exit the starting square (first grid crossing), number of grid lines that were crossed, number of defecations, the number of escape attempts (flying), and whether other behaviours were performed. Other behaviours included head-shaking, wing-flapping and preening. Two video cameras were positioned 3 m above the test arena, 1 m apart, to provide an overhead view of bird movement in the test arena (Platinum HD-TVI Turret Camera, model CMHT1823 eco, LTS). Bird movements were observed on a video monitor located approximately 3 m from the test arena, to avoid human interference with test responses. Activity levels were quantified by using a 3 × 3 grid marked on the floor, and the quantity of vocalisations made by each bird was recorded using an ascending scale from 0 (no vocalisations) to 5 (continuously vocalising with one or no pauses). The volume of vocalisations was recorded by the use of a 3-tiered scale, allocating the vocalisations made in each 30 s interval a score of 'silent', 'soft' or 'loud'. Tables describing the vocalisation scoring systems are provided in Hartcher *et al.*, 2015a). The TI test and OFT were conducted between 35 and 42 weeks of age, and all focal birds from each pen were tested on the same afternoon from 1300 h. Each focal bird was also weighed and feather-scored at the time of testing.

In-situ behaviour observations

In addition to the feather-pecking observations, other behaviours were recorded during the *in-situ* behaviour observations at 43 and 44 weeks of age; dust-bathing, ground-scratching, aggressive pecking, and ground-pecking. Definitions of these behaviours were based on descriptions in the ethogram by Nicol *et al*. (2009). Dustbathing was recorded when birds lay on the floor to scratch the litter, open their wings and disperse wood shavings through their feathers. Ground-scratching was performed where birds assumed a standing, slightly crouching posture and raked their legs in a backwards motion across the litter, prior to moving backwards to inspect the ground at which they had scratched. Ground-pecks were performed to the floor of the pen, and aggressive pecks were vigorous pecks directed at the head region (Nicol *et al*., 2009).

Statistical Analyses

The fixed model for all analyses was:

Environmental enrichment (yes/no) + Plumage damage in a pen (yes/no) / Bird classification (pecker, victim or control)

Behaviours in the OFT which were recorded as binomial data (whether the bird defecated, flew or performed other behaviours including head-shaking, wing-flapping and preening) were analysed using binomial Generalised Linear Mixed Models in GenStat (15th edition). Activity levels in the OFT were analysed with a Generalised Linear Mixed Model specifying a Poisson distribution. An ordinal, logistic Generalised Linear Mixed Model was fitted to the ordinal quantity and volume data. The model was fitted using ASReml 3.0 (VSN International, Hemel Hempstead, UK) and pairwise comparisons were conducted where there were significant associations. The pen and observer were the random effects for all OFT analyses. Bodyweights were log-transformed to achieve a normal distribution. A Linear Mixed Model was then run using GenStat (15th edition) with pen in the random model. The OFT and TI tests were terminated at 300 s and 180 s, respectively. Due to censoring, the duration of TI, time to perform the initial step and time to exit the starting square in the OFT were analysed using Cox's proportional hazards model using the 'survival' package in R, version 3.0.1 (R Core Team, 2015). Pen and observer were specified as the random effects. P values were treated as significant if less than 0.05. Since a number of different statistical tests were performed on a variety of outcome variables, the reader may choose to apply the Bonferroni test to correct for multiple comparisons. In this experiment, there were four pairwise comparisons for each statistical test which yielded a P value < 0.05, when data were analysed with bird classification as the fixed effect. Therefore, each P value reported in the results may be accepted as indicating a statistical difference if less than 0.05 as is convention, or less than 0.01, if applying the Bonferroni test. On the pen level, only two pairwise comparisons are made, so the P value may be accepted as different when < 0.05.

RESULTS

During *in situ* behaviour observations, birds from enriched pens tended to receive less SFP (0.94% vs. 1.93% of the observed time budget, $P = 0.06$). There were no effects on a pen level (plumage damage or environmental enrichment) or individual bird level (classification) on the other behaviours which were recorded; ground-scratching, ground-pecking, aggressive pecking and dustbathing, or on body weight (all $P > 0.1$). In the OFT, peckers made more vocalisations (Figure 1, $P < 0.001$), and vocalised more loudly (Figure 2, $P < 0.001$) than victims. Control birds did not differ from peckers or victims in their vocalisations (all $P > 0.1$).

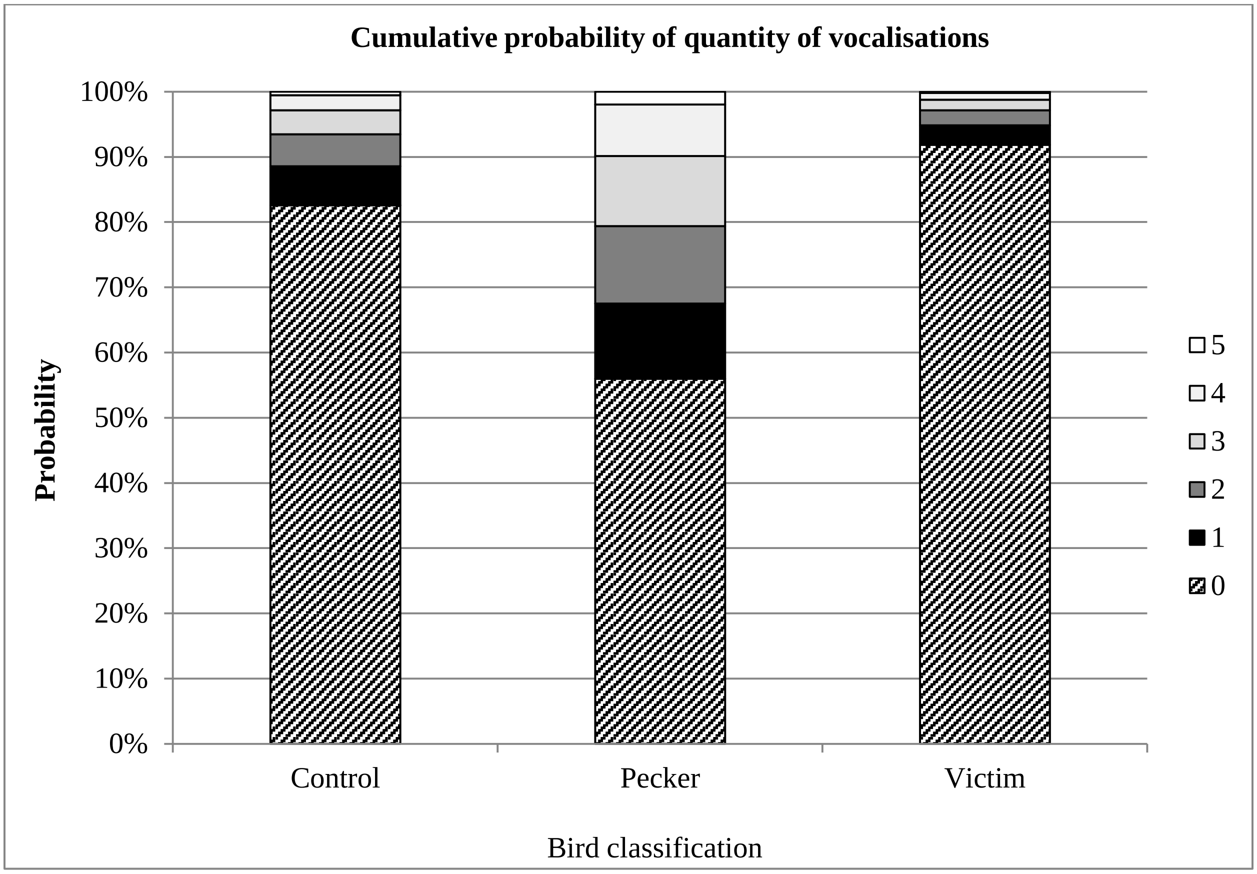

Figure 1. Cumulative probabilities of the quantity of vocalisations (on an ascending ordinal scale of 0 to 5) made by focal birds (n = 70) during a 5 min open-field test, as analysed using an ordinal logistic generalised linear mixed model (*P* < 0.001).

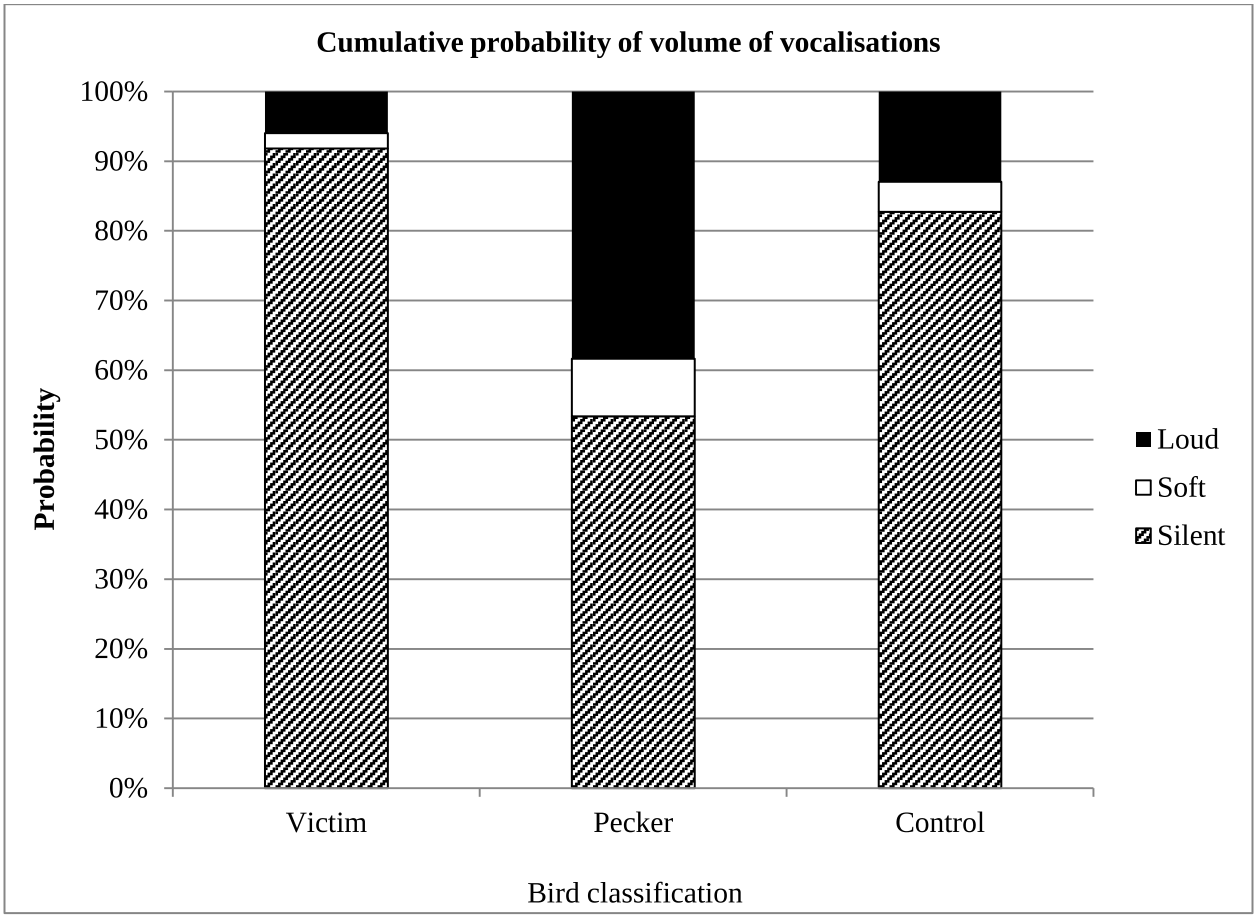

Figure 2. Cumulative probabilities of the volume of vocalisations (on a 3-tiered scale, of silent, soft and loud) made by focal birds (n = 70) in a 5 min open-field test, as analysed using an ordinal logistic generalised linear mixed model (*P* < 0.001).

Peckers also performed more flying, or ‘escape attempts’ during the OFT (16.0 vs 1.2 vs 0.04 for peckers, victims and controls, respectively, $P = 0.03$). There was no effect of plumage damage in a pen or enrichment on the quantity or volume of vocalisations ($P > 0.1$). Birds from enriched pens had shorter latencies to perform their first step (98.4 vs 131.7 s, $P = 0.04$) in the OFT. There were no other effects of plumage damage, EE or bird classification on variables recorded during the OFT (all $P > 0.1$).

Victims had longer TI durations than peckers ($P < 0.001$), and tended to have longer latencies than controls ($P = 0.08$), (a term plot illustrating the hazard rate is presented in Figure 3, with average latencies presented in Figure 4). The proportion of birds that were censored (TI > 180 s) were 0%, 28% and 55% for peckers, controls and victims respectively. On the pen level, there were no effects of plumage damage or enrichment on the duration of TI ($P > 0.1$). If the Bonferroni test is applied to the results, the results which would still be considered significant are the observations of SFP, the feather-scores, the effect of bird classification on vocalisations in the OFT and the effect of bird classification on TI duration (all $P < 0.01$).

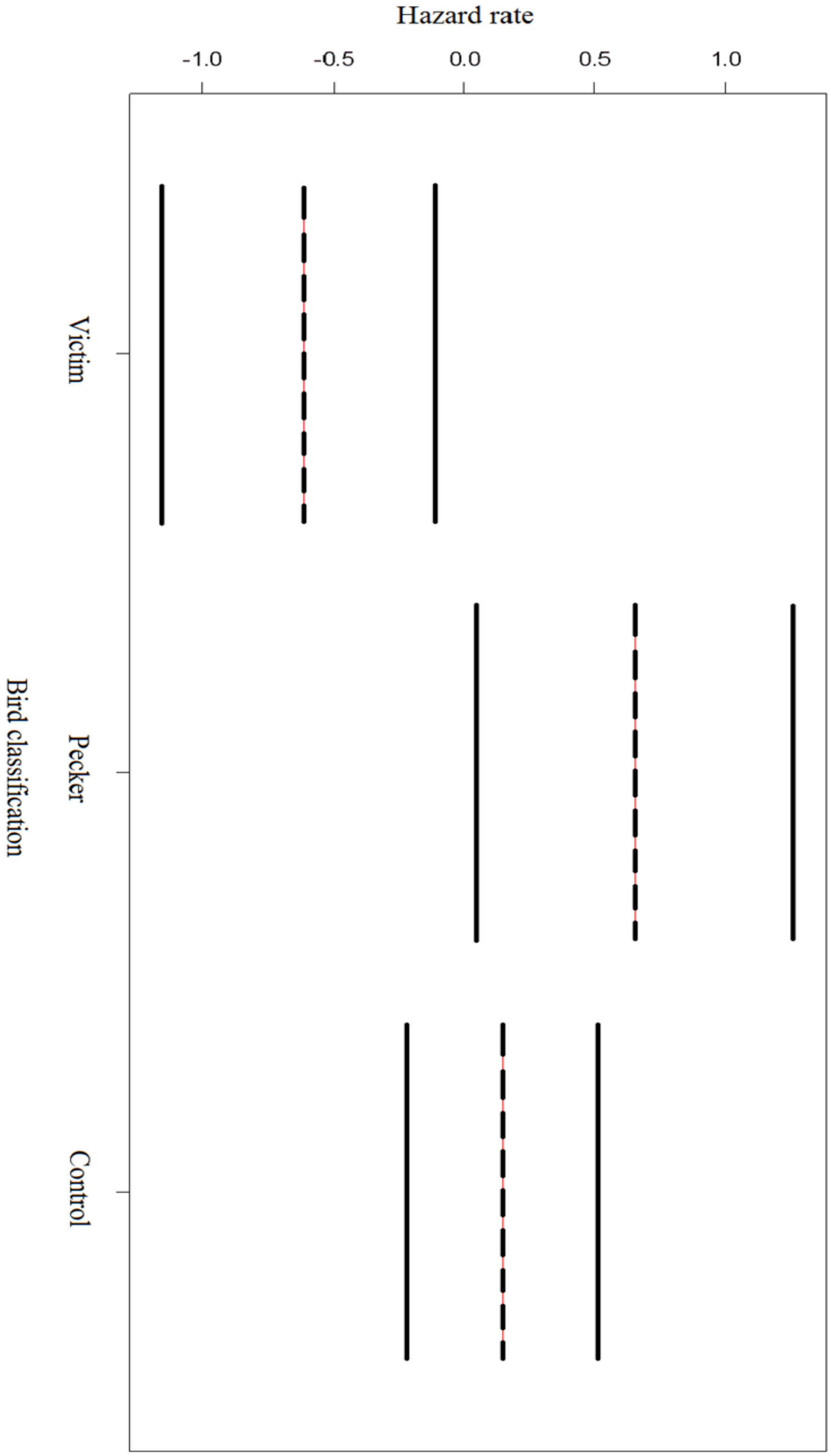
Hazard rate
-1.0
-0.5
0.0
0.5
1.0
Bird classification
Victim
Pecker
Control

Figure 3. Term plot with means and standard errors, illustrating the effect of bird classification (n = 70 birds) on the duration of tonic immobility (*P* < 0.001), as analysed using Cox's proportional hazard test for survival analyses with censoring (at 180 s).

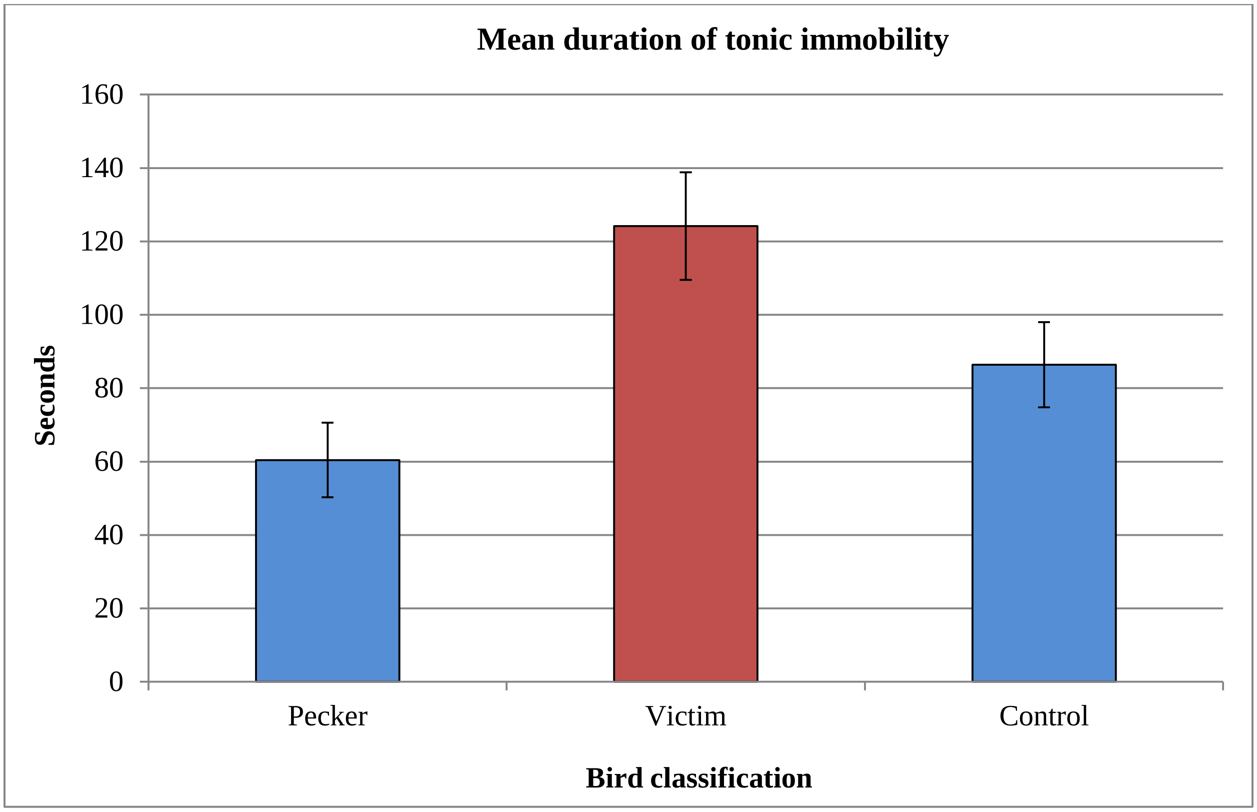

Figure 4. Mean durations of tonic immobility per classification (n = 70 birds).

DISCUSSION

Tonic immobility and open-field tests

Victims had the longest TI durations. This indicates that birds with the most plumage damage were also more fearful, and supports previous studies which found positive associations between TI and plumage damage (Blokhuis and Beutler, 1992; Johnsen *et al*., 1998). This result contrasts with Vestergaard *et al*. (1993), who found that the most fearful birds as assessed by a TI test at 42 weeks of age also performed the most feather-pecking, and Jones *et al*. (1995) found no differences between high and low feather-pecking lines in their responses to a TI test. However, Vestergaard *et al*. (1993) observed feather-pecking associated with dust-bathing without distinguishing between GFP and SFP.

In the present experiment, when individual birds were identified and categorised, there was an effect on TI reactions. However, whether or not a pen had plumage damage had no effect on TI. This highlights the important role that individual variation plays in the relationship between fearfulness and SFP. Peckers made more vocalisations and escape attempts in the OFT, and had shorter latencies in the TI test than victims. Hence it would appear that SFP birds exhibited more proactive responses to the tests. Previous studies suggested that birds which perform SFP may exhibit proactive coping styles in response to fearful stimuli (Rodenburg *et al*., 2004b; Jensen *et al*., 2005; de Haas *et al*., 2010).

Responses in an OFT may be interpreted as proactive or reactive coping styles in response to fearful stimuli, or as the opposing tendencies to avoid detection by predators and the need for social reinstatement. For example, a bird with a higher number of escape attempts in an OFT may be expressing a proactive coping style, or a greater need for social reinstatement (Forkman *et al*., 2007). Some studies have theorised that vocalisations and escape attempts in an OFT are indicative of underlying social motivation (Gallup Jr *et al*., 1980; Jones *et al*., 1995; Forkman *et al*., 2007). Indeed, Marx *et al*. (2001) concluded that social isolation is vocally expressed in young chicks as distress calls, although hens may have a lower need for social reinstatement than young pullets and chicks (Rodenburg *et al*., 2003). Peckers had shorter TI durations, vocalised more and also exhibited more escape attempts in the OFT. This indicates that the peckers were less fearful and had a higher need for social reinstatement.

Environmental enrichment

It was hypothesised that birds from enriched rearing environments would exhibit less fearful reactions in the OFT and TI test, and also exhibit less SFP. While there was no effect of EE on feather-scores, birds from enriched pens tended to receive less SFP than non-enriched pens. Birds in the pens with no enrichment may have performed SFP with greater efficiency and higher rates of feather removal, potentially linked with higher feather-eating. However, feather-eating was not recorded in the present experiment. The birds from enriched pens had shorter latencies to perform their first step in the OFT, which may indicate reduced fearfulness. However, there were no differences in the duration of TI. Daigle *et al*. (2014) found dissimilar results, where birds that had been provided with enrichment at 22 weeks of age (hay bales or hay contained in plastic boxes) were slower to vocalise during a manual restraint test and also performed fewer struggles, suggesting that these birds were more fearful than their counterparts which had not been provided with EE.

The first 10 days of life may be an important and influential developmental time period for behaviour later in life (Johnsen *et al*., 1998; Janczak and Riber, 2015). Bird behaviour is also strongly influenced by the current environment (Nicol *et al*., 2001; De Jong *et al*., 2013). In the present experiment, although enrichment was provided during rearing, it was not provided in the first 12 days, and whole oats and differentiated litter depth ceased more than 10 weeks prior to testing. The birds may have been more influenced by their current environment. When the provision of EE ceased, litter depth was not decreased in the enriched pens, but built up in the non-enriched pens. However, the provision of whole oats ceased at 20 weeks of age, hence around the point of lay. Disruption at this sensitive period may have caused frustration or stress, and contributed to a subsequent development in SFP, and a lack of difference between enriched and non-enriched pens in plumage condition later on. Replication in the present experiment was limited; hence results should be extrapolated and discussed with this consideration in mind. However, the results are in line with those from Hartcher *et al*. (2015b) which was conducted on the same flock of birds, where the EE did not have an effect on plumage damage later in life.

In-situ behaviour observations

Not surprisingly, birds in pens with plumage damage performed (and received) more SFP. These birds also performed more GFP. Similarly, peckers performed more SFP and tended to perform more GFP. It was interesting that the birds performing more SFP also performed more GFP, as it is thought that SFP and GFP are distinct behaviours with different underlying causes (Hughes and Buitenhuis, 2010). Despite the two behaviours resulting in very different outcomes to the recipient bird, results from the present study suggest that there may be an association between the two behaviours, where birds which are more likely to perform SFP may also be more likely to perform GFP. This is in agreement with Rodenburg *et al*. (2004b) who found a correlation between SFP and GFP within age classes, and with van Hierden *et al*. (2002) who found that a high feather-pecking line expressed more SFP and GFP than a line selected for low feather-pecking.

Some studies have suggested that GFP is a normal behaviour, which plays an important role in social exploration and the maintenance of social relationships in chicks (Riedstra and Groothuis, 2002). SFP is not typically thought to be related to sociality. However, vocalisations in the OFT may be interpreted as reflective of social motivation, and also related to SFP (Jones *et al*., 1995). In this study, the peckers vocalised more in the OFT, potentially indicating a higher motivation for social reinstatement. The relationship between GFP and SFP is currently unclear. It is important in behaviour studies and observations that these two behaviours are viewed and categorised separately, in order to facilitate the collection of accurate information and the correct interpretations of behaviour (Newberry *et al*., 2007; Rodenburg *et al*., 2013).

In the present experiment, pens that received more SFP during observations also had more plumage damage, but there was no difference in the likelihood for birds to receive SFP based on their classification. This suggests that when SFP was present in a pen, it was widespread throughout the pen and all birds were targeted similarly. This contrasts with the feather-scores where peckers had fewer damaged body areas than victims. Hence, although all birds may have been targeted equally for SFP, the victims suffered more damage as a result. This may be due to bird responses when targeted for SFP. Birds with the least plumage damage in a flock may possess behavioural characteristics that predispose them to less plumage damage. These behavioural characteristics may include such as establishing greater inter-bird distance when approached, or performing retaliatory pecks to SFP. Avoidance or retaliatory behaviours in response to SFP may be more likely in birds with lower levels of fearfulness as estimated in the tonic immobility test. The differences in the extent of plumage damage were probably due to behavioural rather than physical characteristics, as there was no association between bird classification and body weight. However, other physical characteristics were not measured such as comb and wattle colour or size, bird height etc. The lack of difference in bodyweight contrasts with previous studies that found associations between bodyweight and SFP (Bessei *et al*., 1999).

There was no effect of bird classification or the presence of plumage damage in a pen on the expression of ground-pecking or ground-scratching. This contrasts with previous findings which found relationships between foraging behaviour and the expression of SFP (Blokhuis and van der Haar, 1992; Johnsen *et al*., 1998). Many studies have reported that SFP represents a redirection of foraging pecks to the feathers of conspecifics (Blokhuis and Arkes, 1984; Huber-Eicher and Wechsler, 1997; Dixon *et al*., 2010; Gilani *et al*., 2013), whereas some have found a positive association between SFP and foraging (Bilčík and Keeling, 2000; Newberry *et al*., 2007). Similar to the findings of the present experiment, Blokhuis and van der Haar (1989) also found no differences in the frequency of ground-pecking, despite differing levels of plumage damage in birds reared on wire or litter. The relationship between SFP and foraging is complex and warrants further investigation.

Selection methods

Victims performed a small amount of SFP, and peckers exhibited some plumage damage. Hence victimisation does not exclude the possibility for birds to also perform SFP (Daigle *et al*., 2014). The likelihood of performing SFP may be greater in groups of birds in which plumage damage and SFP are widespread, due to the social transmission of the behaviour (Rodenburg *et al*., 2013). Daigle *et al*. (2015) categorised birds as feather peckers, victims, neutrals, or feather pecker-victims based on behaviour observations. While some birds remained consistent, approximately half of the birds in that study exhibited inconsistent behavioural profiles over a 16 week period, transitioning between categories. In the present experiment, birds were categorised and testing was performed shortly afterwards, giving little time for birds to transition to a different behavioural profile, or ‘classification’. Additionally, observations during weeks 43 and 44 found that peckers performed more SFP, and victims had the most plumage damage at 43 weeks of age. These results indicate that the classifications were largely representative of bird behaviour.

CONCLUSIONS

Victimised birds had longer durations of TI, and poorer plumage condition. Peckers exhibited more active responses in the OFT in the form of vocalisations and escape attempts. Hence peckers may be more socially motivated, while victimised birds may have higher levels of fearfulness. These results provide important information about birds' underlying behavioural states, which contribute to the current understanding of the causes of SFP on an individual bird level. Ultimately, an improved understanding of the behavioural characteristics of birds that perform SFP will help to develop methods which control its expression.

ACKNOWLEDGEMENTS

The Australian Egg Corporation Limited and the Poultry Cooperative Research Centre, established and supported under the Australian Government's Cooperative Research Centres Program, are gratefully acknowledged for providing research funding.

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CHAPTER 6: The association between plumage damage and feather-eating in free-range laying hens

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Published in: *Animal* (2016)

10:854-862

OVERVIEW OF CHAPTER 6

In chapters 3 and 5, whilst conducting *in situ* behaviour observations, experimenters observed that many of the severe feather-pecks resulted in the ingestion of the pulled feather. Conjecturally, it appeared that birds may have performed severe feather-pecking for the purpose of removing the feathers and ingesting them. Severe feather-pecking, feather-pulling, and feather-eating were often performed with vigour and rapidity. Studies have suggested a relationship between feather-eating and severe feather-pecking. However, as outlined in chapter 2, more information is required on this association. The motivation for birds to perform feather-eating is not understood, nor why certain birds and body areas can be targeted for SFP, and if this may be related to their feather characteristics and attractiveness for ingestion. It has also yet to be determined whether feather-eating may act as a predictor of severe feather-pecking. The first experiment in chapter 6 therefore compared feather-eating in pens of birds expressing severe feather-pecking, with pens of birds with very little plumage damage and severe feather-pecking. The second experiment was conducted to investigate the predictive relationship. Feathers from different birds and different body areas were also compared for their attractiveness for ingestion.

ABSTRACT

Severe feather-pecking (SFP) persists as a highly prevalent and detrimental behavioural problem in laying hens (*Gallus gallus domesticus*) worldwide. The present experiment investigated the association between feather-eating and plumage damage, a consequence of SFP, in groups of free-range, ISA Brown laying hens. Single feathers were placed on the floor of the home pens. Feathers were sourced from seven different birds. Fifty birds in six pens with extensive plumage damage were compared to birds in six control pens with little plumage damage at 41 to 43 weeks of age (n = 12 pens, 600 hens). Birds in pens with extensive plumage damage ingested more feathers ($P = 0.02$), and also showed shorter latencies to peck at ($P < 0.001$), and ingest feathers ($P < 0.001$). Birds ingested feathers from a bird in the free-range facility, in which the testing took place, more quickly than from a bird housed in a separate cage facility ($P < 0.001$). A second experiment investigated the predictive relationship between feather-eating and plumage damage. Feathers were presented to 16 pens of 50 pullets prior to the development of plumage damage, at 15 weeks of age, and then to the same hens after plumage damage had become prominent, at 40 weeks of age. Birds had a higher probability of ingesting feathers ($P < 0.001$), pecked feathers more times ($P < 0.001$), and also pecked ($P < 0.001$) and ingested ($P < 0.001$) the feathers more quickly at 40 than 15 weeks of age. There was a trend for an interaction, where birds pecked feathers from the rump more times than feathers from the back at 40 weeks of age ($P = 0.06$). However, a lack of variability in plumage damage between pens in this experiment precluded investigation of the predictive relationship. The results from the present study confirm the association between feather-eating and plumage damage, and suggest that birds may prefer feathers from particular body areas and from particular hens. Future experiments should focus on elucidating whether feather-eating may act as a predictor of SFP.

Key words: feather-pecking, feather-eating, laying hen, behaviour, plumage damage

INTRODUCTION

SFP is a detrimental behaviour, whereby birds vigorously peck at, pull, and sometimes remove and ingest feathers from other birds (Blokhuis and Arkes, 1984; Savory, 1995; Gunnarsson *et al.*, 1999). It has been identified as the most serious welfare risk for laying hens (Bestman *et al.*, 2009; Bessei and Kjaer, 2015). SFP may be more difficult to control in non-cage housing systems due to large group sizes. Non-cage housing systems, including free-range, have been increasing in prevalence in countries including Australia (Rault *et al.*, 2013). Despite over five decades of extensive research on the topic, SFP persists as an unresolved problem in the poultry industry worldwide (Harlander-Matauschek and Rodenburg, 2011; Bessei and Kjaer, 2015).

The dominant theory proposes that SFP is a form of redirected pecking, whereby birds redirect pecking behaviour normally associated with foraging and food-searching, away from the environment and towards conspecifics instead (Blokhuis, 1986; Huber-Eicher and Wechsler, 1998). This may occur in environments which lack adequate stimuli (Hughes and Duncan, 1972; Blokhuis, 1986; Bessei and Kjaer, 2015). However, birds also express SFP in housing systems which contain plentiful environmental stimuli, such as deep litter and free-range systems (Bessei and Kjaer, 2015). Although environmental influences are important (Rodenburg *et al.*, 2013), other factors have been identified as increasing the risk of SFP, including genetic influences and individual bird differences (Jensen *et al.*, 2005; Wysocki *et al.*, 2010). In particular, previous experiments have reported a positive relationship between feather-eating and SFP (McKeegan and Savory, 1999; Harlander-Matauschek *et al.*, 2006a; Harlander-Matauschek and Häusler, 2009).

Specific body areas, such as the rump and back regions, are often targeted when birds perform SFP. This can cause certain areas on birds' bodies to become denuded, while other areas remain fully-feathered (McKeegan and Savory, 1999; Harlander-Matauschek *et al*., 2007b). Similarly, particular birds in a flock may become targeted for SFP and experience extensive denuding and wounds, while other birds in the same flock remain undamaged (McKeegan and Savory, 2001). It is not known why some birds in a flock become the targets for SFP.

The present study is comprised of two separate experiments. Experiment 1 investigated the association between feather-eating and plumage damage, on a group level. Studies in this area have focused on the individual bird level, although it is also important to collect information on feather-eating and SFP on a group level. Experiment 1 assessed feather-eating by presenting single feathers on the pen floor to pens of free-range ISA Brown laying hens with extensive plumage damage compared to control pens, in which there was very little plumage damage. Plumage condition is highly correlated with the amount of SFP received (Bilcik and Keeling, 1999), and feather-scoring is often used to assess the level of SFP in flocks of laying hens (Hughes and Duncan, 1972; Gunnarsson *et al*., 1999; Ramadan and Von Borell, 2008). The best age at which to feather-score was deemed to be 40 weeks by Yamak and Sarica (2012). The three groups of birds in the present study were therefore feather-scored at 40, 41 and 44 weeks of age. This was also the time the most plumage damage was evident (plumage damage appeared cumulative), as the groups were depopulated at these ages.

An additional aim of experiment 1 was to investigate responses to feathers sourced from different birds. Feathers were collected from birds housed in a separate cage facility, from birds housed within the same shed as the present testing took place, and from the floor of the shed in which testing took place. It was hypothesised that pens of birds with plumage damage would have shorter latencies to peck at and ingest single feathers presented to them, and would also be more likely to ingest feathers than pens of birds with little plumage damage. Feathers taken from different birds were expected to elicit different reactions.

Experiment 2 aimed to investigate the temporal relationship between feather-eating and plumage damage, by presenting feathers to pens of birds prior to, and following, the development of plumage damage. In addition, feathers were taken from different areas of the body, to investigate whether birds show preferences for feathers from particular body areas. Experiment 2 also included quantification of feathers on the pen floors, and novel object tests in the home pens. Novel object tests were to investigate whether the placement of feathers by experimenters may have caused stimulus enhancement, and whether some pens may have been more fearful than others (Forkman *et al*. 2007), which could affect bird responses to the introduction of feathers to the pen.

It was hypothesised that birds which developed SFP would demonstrate higher rates of feather-eating prior to the development of plumage damage and SFP, and also show preferences for feathers from different body areas. Since the rump was the first body area to exhibit feather loss attributed to SFP, it was expected that birds may prefer to ingest feathers from the rump. The initial aim of both experiments was to discern whether there was significant variation between pens of birds in their plumage damage, in order to then use plumage damage as a predictor variable for outcome variables including feather-eating. ISA Brown laying hens were used due to their popularity for egg production, their high rates of SFP in commercial conditions, and their sensitivity to the environment compared to white hens (de Haas *et al*., 2014a).

MATERIALS AND METHODS

Experiments 1 and 2 complied with the University of Sydney Animal Ethics Committee approved protocols and with the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2004).

Experiment 1

Birds, Husbandry and Housing

Two groups of 300 non-beak-trimmed ISA Brown laying hens were used (n = 600), housed in 12 pens, in the same housing facility, one year apart. Both groups were reared in indoor, non-cage facilities, in 16 pens of 50 birds. Fifty birds were then housed in each pen in the free-range laying facility. Pens measured 1.83 m × 3.25 m in a naturally ventilated, uninsulated shed with wood shavings spread on concrete floors. Each pen had continuous access to an outdoor range area measuring 1.83 m × 10 m from 26 weeks of age in the first group, and 20 weeks of age in the second group, relative to the age at which they started laying. The total space for the 50 birds in each pen was 24.25 m^2, giving a stocking density of 2.1 birds per m^2 including the range area. Range areas were separated from each other by wire fences, 2.1 m high, with wire netting over the top to protect against predators; indoor pens were separated by wire as well as shade cloth to provide visual barriers.

All birds were of the same strain, originated from the same commercial hatchery and parent stock, and housed in the same environment with the same feed and water provisions. Water was provided via one red bell drinker and a commercial mash feed (Vella Stock Feeds, Sydney, Australia; Table 1) was available *ad libitum* via a metal bell feeder suspended from the ceiling in each pen. Continuous lighting was provided for 15 h in every 24 h via fluorescent tubes, with some natural light entering the shed through shed doors and pop-holes, giving an average light intensity of 52.0 lux throughout the shed. Light levels were measured using a Tonda j Digital Lux Meter (Model number: LX-1010B, Instrument Choice, Laboratory Equipment Supplier, Dry Creek, South Australia). Each pen contained a perch unit consisting of five timber perch rungs, 4 cm wide by 125 cm long, at five different heights. Distance from the floor surface to the top of the lowest perch was 25 cm, with each perch (top surface) 20 cm higher than the perch below it (based on the vertical profile). Each perch was 20 cm apart (based on the horizontal profile).

Table 1. Nutrient composition of the pullet grower feed and layer feed provided to ISA Brown laying hens during the experimental period.

	Pullet grower feed		Layer feed	
Typical Analysis	As Fed	Dry Matter	As Fed	Dry Matter
Energy KCAL/kg	2300	2614	2500	2841
Protein (%)	16.00	18.18	16.50	18.75
Calcium (%)	1.31	1.90	3.45	3.92
Phosphorus (%)	0.48	0.55	0.48	0.55
Fibre (%)	6.70	7.61	5.38	6.11
Fat (%)	3.84	4.36	5.52	6.27

Single feather test

Single feathers were presented to pens of birds at 43 weeks of age in the first group. Feathers were collected from the floor of the housing facility and from three ISA Brown laying hens (n = 4) of a similar age to the study hens, but housed individually in conventional cages in a separate facility. The same testing methodology was repeated one year later on a second group of free-range ISA Brown laying hens, at 41 weeks of age. For this flock, feathers were collected from three birds in three different pens (n = 3) within the same housing facility as the tested birds, from pens that were not being tested (total number of birds from which feathers were taken n = 7). Feathers were cut from the belly and legs of all birds (see figure in Bilcik and Keeling, 1999). All feathers used for testing were short and downy, of the same dimensions, approximately 3 cm in length. Feathers were not plucked from live birds.

Single feathers were placed on the litter in the centre of each home pen. One observer entered the test pen, holding a feather between thumb and forefinger at arm’s length for a period of approximately 5 s to give birds an opportunity to view each feather prior to placement. Following this, the observer slowly placed the feather on the litter in the centre of the pen away from any obstructing elements such as the perches, nest boxes or feeders. Feathers were placed in a central location in all pens, which was deemed to be most easily visible to all birds within the pens (Figure 1).

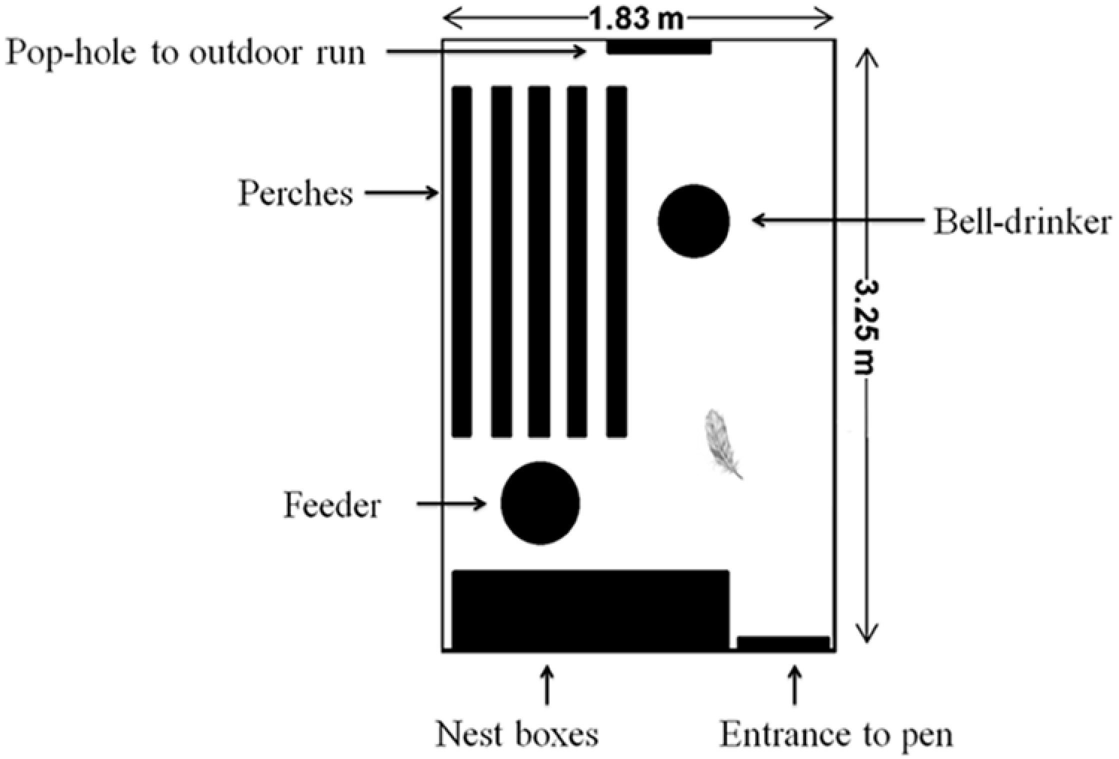

Figure 1. Individual housing pen layout, in which 50 ISA Brown laying hens were kept. The image of the feather denotes the approximate feather placement site in each pen prior to the commencement of each loose individual feather test, in which the latency to peck and ingest the individual loose feather were recorded. Figure modified from Hartcher *et al.* (2015).

Immediately after each feather had been placed, the observer took one step back from the feather towards the door through which they had entered, and stood stationary to record responses to the feather. Birds were habituated to regular human presence in the pens. The variables recorded were the latency to the first peck at the feather by any bird, the latency to ingestion of the feather by any bird, and whether or not the feather was ingested. The test was terminated at 30 s, as preliminary observations showed that if the birds were to react to the feathers that they would do so within 30 s. If the feather had not been ingested at 30 s, it was manually removed from the pen by the observer prior to placing the next feather. Feathers were presented to each pen five times in a row.

Pens were tested in a random order, balanced for plumage damage. Feathers were presented to pens at least 15 times each, on a single day. Pre-existing feathers on the floors of the pens were not removed prior to the commencement of the test. Birds pecked and ingested feathers that were placed on the floor of the pen despite the presence of existing feathers. All test pens had existing feathers on the pen floors. Since there was no variability between the physical aspects of the pens, this was a constant, and did not need to be controlled for in test procedures.

Feather-scoring and behaviour observations

All birds were individually feather-scored at 44 and 41 weeks of age, in groups 1 and 2, respectively. The body areas scored were the neck, back, rump, tail, sides and cloacal region (diagram in Bilcik and Keeling, 1999). The method for feather-scoring was adapted from Tauson *et al*. (2005). Each body area was assigned a score from zero to four where a score of zero denotes perfect plumage condition for that body area, a score of one indicating some feather damage but no bare batches, two denoting some small bare patches, three indicates a body area with extensive feather loss and bare patches, and four signifies a denuded body area. Two methods were used to determine the difference between pens in the amount of plumage damage; the proportion of body areas with plumage damage per bird, and the proportion of birds in each group with plumage damage. This is described later in the statistical analyses subsection.

In-situ behaviour observations were conducted for the first group at 43 to 44 weeks of age. Ten focal birds were selected for observations in four pens (n = 39 due to one death); two pens with extensive plumage damage, and two pens with minimal plumage damage. In the pens with minimal plumage damage, focal birds were selected for observation at random. Random selection involved preselecting areas within the pen and then randomly selecting a bird by sight, and then selecting two birds over for testing, thereby avoiding any bias or the selection of birds which may have been slower or less fearful of humans, and easier to catch. In pens with extensive plumage damage, focal bird selection attempted to balance for plumage damage and SFP. Three birds per pen were selected due to extensive plumage damage, and three were selected if they were seen to perform SFP, plus four others, which did not exhibit extensive plumage damage nor SFP behaviour (n = 10 focal birds per pen). Hens performing

SFP were selected as focal birds when they were observed to perform more than four severe feather-pecks in a 10 min period.

The order of pens and birds observed was randomised to account for the effect of time of day. Birds were marked with coloured spray-paint on the tail or wings for identification, and each observed for a 2-min period, which was classified as one observation session. Twenty-seven observation sessions were conducted for each bird, giving a total of 54 mins for each of the 39 focal birds. Each 2-min observation session was divided into 30 s intervals in which behaviours were recorded binomially as ‘present’ or ‘absent’. Behaviours which were recorded included SFP (performed and received), gentle feather-pecking (performed and received), ground-pecking, ground-scratching, dustbathing, and aggressive pecking (performed and received). Definitions of these behaviours were based on descriptions in the ethogram by Nicol *et al*. (2009). Gentle feather-pecking was recorded when a peck did not appear to involve the grasping or pulling of feathers and was not administered with force. SFP was administered with force, and was recorded if a peck involved the grasping or pulling of feathers, or both. Dustbathing was when birds lay on the floor to scratch the litter, open their wings and disperse wood shavings through their feathers. Ground-scratching was performed when birds assumed a standing, slightly crouching posture and raked their legs in a backwards motion across the litter. Ground-pecks were performed to the floor of the pen, and aggressive pecks were vigorous pecks directed at the head region (Nicol *et al*., 2009).

Statistical Analyses

Feather-scores were analysed using the Generalised Linear Mixed Models procedure in GenStat (15th edition, VSN International, Hemel Hempstead, UK), run with a binomial distribution. For the first analysis, the number of birds per pen with plumage damage (yes or no) was the response variate. For the second analysis, the response variate was the number of body areas with plumage damage per bird. The fixed model for both analyses was the pen with year as the random effect.

As reported in the results section, half of the pens were identified as having extensive plumage damage and half with little plumage damage. Two subpopulations were then created; pens with extensive plumage damage and pens with little plumage damage. All further analyses were then run with these subpopulations as the fixed effect, and the year as the random effect. Hence the fixed effect was the presence of extensive plumage damage in a pen (yes or no). Data from behaviour observations were also analysed using the Generalised Linear Mixed Models procedure in GenStat (15th edition) run with a binomial distribution. The response variate was the number of time periods in which a behaviour was observed (i.e. ground-pecking or ground-scratching etc.). The total number of observation periods was the denominator. Hence behaviours were analysed as a proportion of observation sessions in which they were recorded as present. Pen and bird were the random effects, as there were multiple observations per bird.

Binomial data for the probability of feather ingestion were also analysed using the logistic Generalised Linear Mixed Models procedure in GenStat (15th edition), run with a binomial distribution. Feather ingestion (yes or no) was the binomial outcome variable. Latencies to peck and ingest feathers were analysed using Cox’s proportional hazards model using the ‘survival’ package in R, version 3.0.1 (R Core Team, 2015). Survival analyses were used due to censoring at 30 s. Hence the times to peck and ingest feathers in the 30 s are presented as hazard rates. The bird from which feathers were collected was a fixed effect (as well as the presence of plumage damage in a pen).

Experiment 2

A total of 800 ISA Brown laying hens were used, housed in 16 pens of 50 birds, in the same free-range housing facility as Experiment 1, one year later (i.e. the year after the second group from experiment 1 was depopulated). This flock was sourced from the same hatchery, and the rearing and laying housing conditions were the same as described for Experiment 1. An ISA Brown laying hen in a separate shed was culled at 15 weeks of age, and feathers were cut from the rump and back. The same methodology for the single feather test was then performed as in Experiment 1. The total number of pecks administered to each feather was also counted. This was conducted in all 16 pens at two time points: prior to the development of plumage damage at 15 weeks of age, and when birds were exhibiting plumage damage at 40 weeks of age. This was in order to elucidate whether birds demonstrated feather-eating prior to the development of plumage damage, or whether feather-eating is associated with plumage damage, but not predictive of it.

Quantification of feather-coverage on the pen floors was performed once prior to the development of plumage damage, at 18 weeks of age, and once when birds were exhibiting plumage damage, at 34 weeks of age. Feathers were quantified using a 0.5 m × 0.5 m quadrat, placed over the middle of the pen (as in McKeegan and Savory, 1999) where the single feather test had been performed. The number of feathers was then counted. A novel object test was also performed. Four different objects were used, where one object was presented to each pen at 15, 19, 33, and 40 weeks of age. Objects were blue cleaning cloths, wooden blocks spray-painted pink as the second object and then re-coloured blue as the third object, and green glass bottles. The novel object was placed in the centre of each pen in the same position as the feathers in the single feather test. The observer then exited the pen and stood quietly on the outside of the pen. After 10 s from placement, the number of birds interacting or interested in the novel object was counted. Interest or interaction with the object was recorded when birds pecked the object or were within an approximate 20 cm radius and looking at, or approaching the object, with their body angled in the direction of the object, appearing to investigate. The number of birds interacting with the novel object was counted every 2 min, for 10 min. Birds were individually feather-scored at 40 weeks of age, using the same scoring procedure as in Experiment 1.

Statistical Analyses

Feathers taken from the rump (n = 128) and back (n = 128) were compared, presented at 15 (n = 160) and 40 (n = 96) weeks of age. Analyses were performed as in Experiment 1, but with body area and week of age as the fixed effects, and the pen as the random effect. Week was included in the random model when analysing for the main effect of body area. The number of pecks to the feathers was analysed using the Generalised Linear Mixed Models procedure in GenStat (15th edition), using a Poisson distribution. The number of existing feathers on the pen floor and the average number of birds interacting with the novel objects were analysed using Analysis of Variance, with pen as the fixed effect and week of age in the random model.

RESULTS

Experiment 1

Single feather test

Birds in pens with plumage damage ingested more feathers (80% vs 26%, $P = 0.02$) and showed shorter latencies to peck at (mean of 4.4 vs 17.9 s, $P < 0.001$) and ingest (mean of 12.9 vs 23.9 s, $P < 0.001$) single feathers than birds in pens with little plumage damage. There was an interaction between the birds from which feathers were sourced (n = 7) and plumage damage in a pen (yes or no), on the latency to peck (Figure 2, $P = 0.07$), and an interaction effect on the latency to ingest (Figure 3, $P = 0.02$) single feathers. There was a significant effect of bird on the latency to ingest (Figure 4, $P < 0.001$) feathers. From the term plot (Figure 3), it appears that the shortest latencies to ingest single feathers occurred in pens in which there were high levels of plumage damage and which were presented with feathers from birds 5, 6, and 7. Birds 1 to 3 were housed individually in the cage facility and their feathers were presented to pens in the first flock, bird 4 denotes feathers collected from the floor of the free-range housing facility and presented to the first flock, and birds 5 to 7 were those in pens within the free-range housing facility in the second flock.

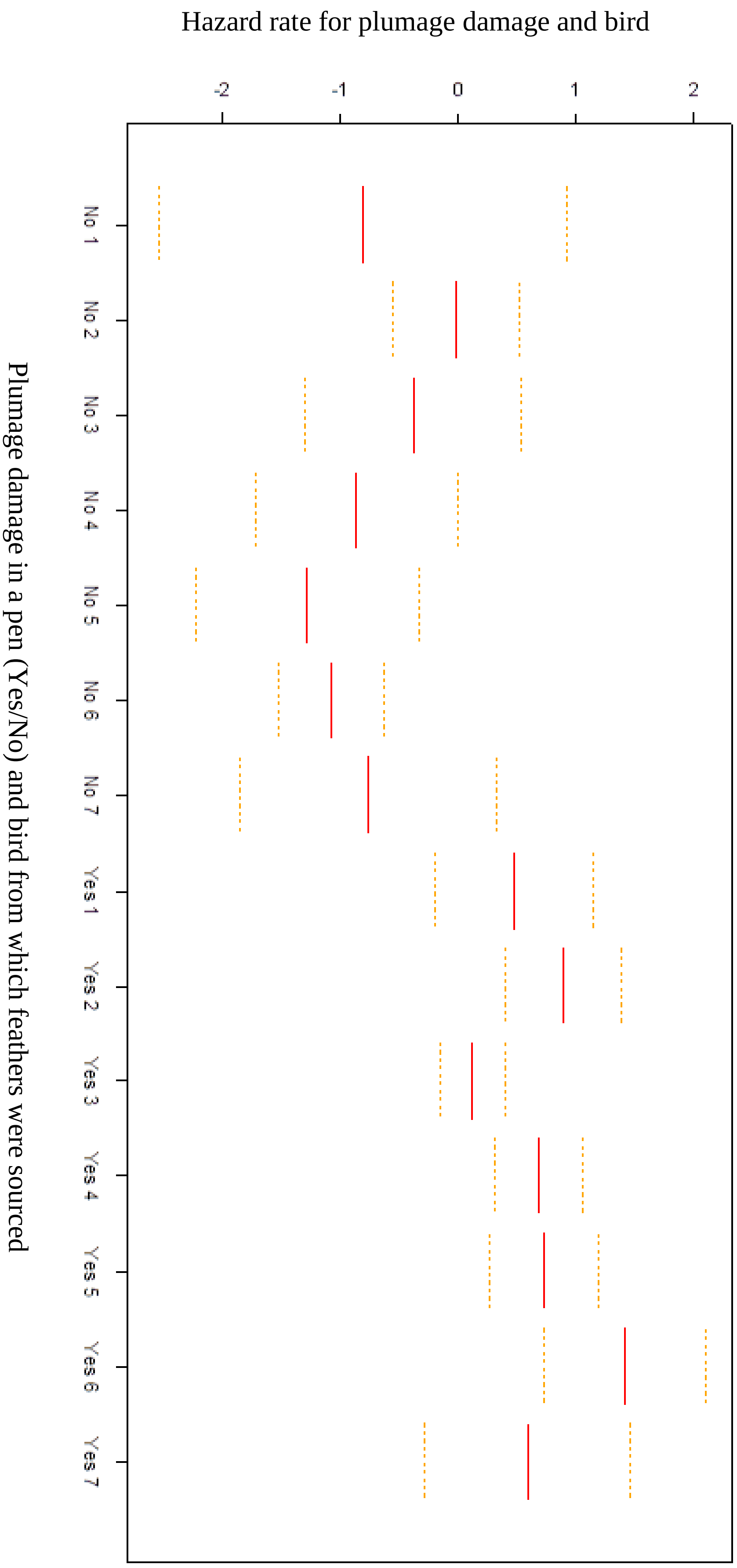
Hazard rate for plumage damage and bird
-2
-1
0
1
2
No 1
No 2
No 3
No 4
No 5
No 6
No 7
Yes 1
Yes 2
Yes 3
Yes 4
Yes 5
Yes 6
Yes 7
Plumage damage in a pen (Yes/No) and bird from which feathers were sourced

Figure 2. Hazard rate for the latency to peck feathers. Term plot with means and standard errors, for the latency of any ISA Brown laying hen within a test pen to peck feathers during single feather tests (*P* = 0.07). Data were analysed using a survival analysis, for the interaction between the hen from which feathers were sourced (1 to 7), and the presence of plumage damage in the test pen (Yes or No).

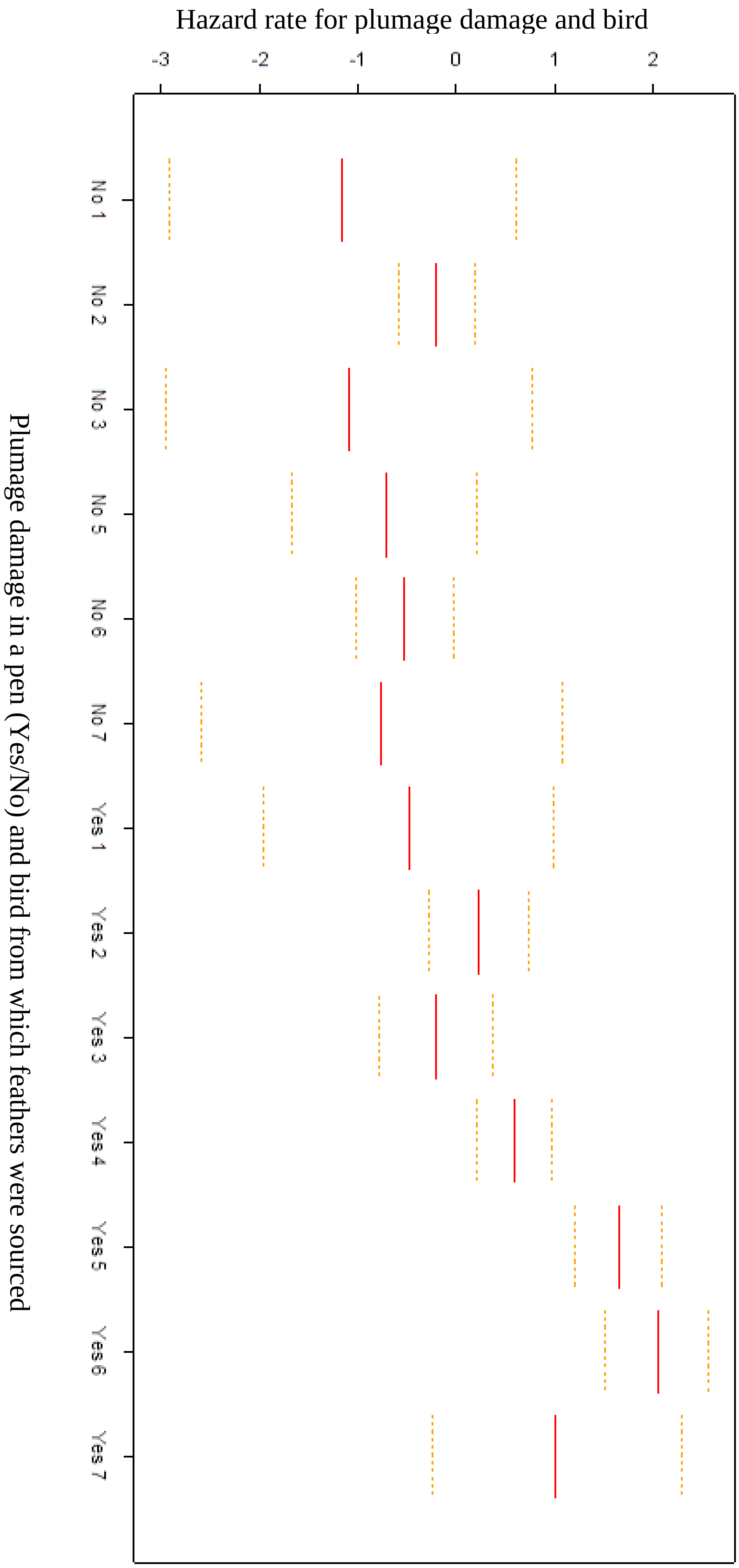
Hazard rate for plumage damage and bird
-3
-2
-1
0
1
2
No 1
No 2
No 3
No 5
No 6
No 7
Yes 1
Yes 2
Yes 3
Yes 4
Yes 5
Yes 6
Yes 7
Plumage damage in a pen (Yes/No) and bird from which feathers were sourced

Figure 3. Hazard rate for the latency to ingest feathers. Term plot with means and standard errors, for the effect of bird and plumage damage in a pen on the latency of a hen to ingest feathers (*P* = 0.02). Data were analysed using a survival analysis, as in Figure 2.

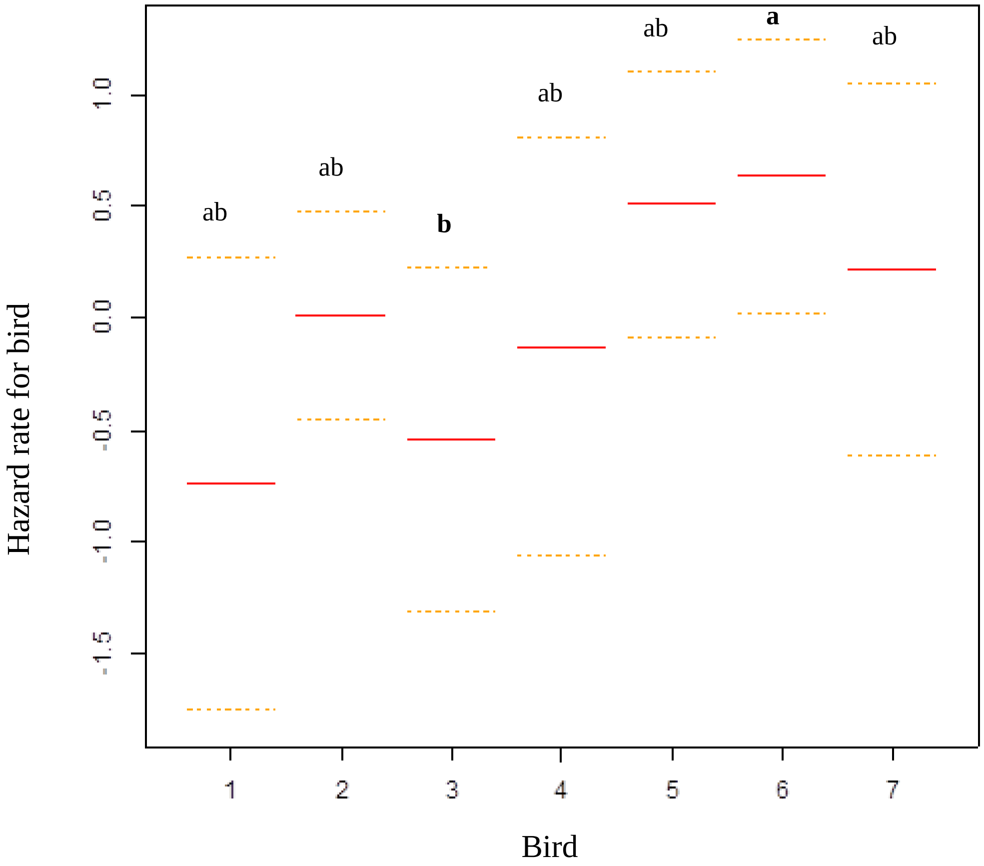

Figure 4. Term plot with means and standard errors for the latency of a hen within the test pen to ingest feathers presented during the single feather test. Data were analysed using a survival analysis to test the effect of the hen from which feathers were sourced (1 to 7). Birds 1 to 3 were housed individually in a separate cage facility, bird 4 denotes feathers taken from the floor of the free-range housing facility in which testing took place, and birds 5 to 7 indicate the birds in pens within the free-range facility. Bars with differing superscripts differ significantly (*P* < 0.001).

Feather-scoring and behaviour observations

There was a difference between pens in the proportion of birds with plumage damage (P < 0.001) and the proportion of body areas with plumage damage per bird (P < 0.001). In half of the pens, more than 80% of birds exhibited plumage damage (average of 88.2%), with more than 20% of their body areas damaged (average of 31.4%). In the other half of the pens, less than 20% of birds exhibited plumage damage (average of 10.5%), with 4% or less of their body areas with plumage damage (average of 2.2%). Hence there were two distinct subpopulations; pens with extensive plumage damage and pens with very little plumage damage, both in terms of number of birds affected, and number of body areas per bird. Consequently, these pens were used for further testing. Analyses were run with these subpopulations as the fixed effect (with or without extensive plumage damage), and results presented in figure 5 (31% vs 2% of their body areas with plumage, P < 0.001, and 93% vs 9% of birds with plumage damage, P < 0.001). Focal birds in pens with plumage damage received more SFP (SFP was observed in 5.4% vs 0.4% of the observation sessions, P = 0.02). Other behaviours (including ground-scratching, ground-pecking, dustbathing and aggressive pecking) did not differ between pens with and without extensive plumage damage (P > 0.1).

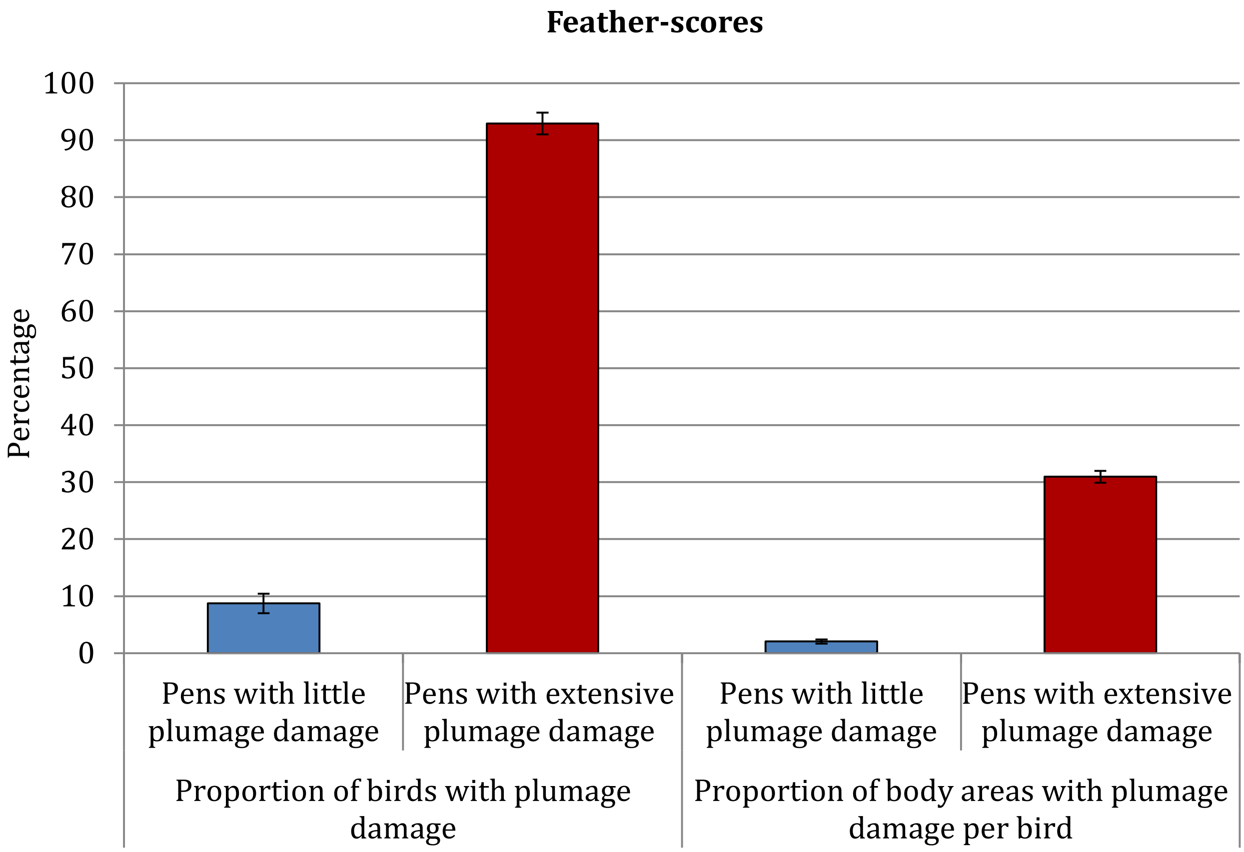

Figure 5. Feather-scores between pens of hens with extensive plumage damage and pens with little plumage damage, as analysed using generalised linear mixed models ($P < 0.001$).

Experiment 2

There was a tendency for birds to peck rump feathers more than back feathers at 40 than 15 weeks of age (interaction: body area*week of age) (Figure 6, $P = 0.06$). At 40 weeks of age, birds were more likely to ingest feathers, (91% vs 5%, $P < 0.001$), pecked feathers more times (average of 2.7 vs 1.4 pecks per feather, $P < 0.001$), and were faster to peck (hazard ratio = 8.5, 95% CI: 5.6-13.1, $P < 0.001$) and ingest (hazard ratio = 30.0, 95% CI: 14.8-60.8, $P < 0.001$) feathers. Birds in pen 14 had a higher proportion of body areas with plumage damage compared to birds in pens 1, 2 and 5 ($P < 0.001$). However, further analyses using plumage damage as a predictor variable was not possible in this experiment. This was due to the lack of difference between more pens, and an unbalanced design if plumage damage in a pen was to be used as a fixed variable in a statistical model. In addition, there were no differences between pens in the number of birds with plumage damage per pen (*P* = 1.0). There were no differences between pens in the number of existing feathers on the pen floor (*P* = 0.79), or the number of birds interacting with the novel objects (*P* = 1.0).

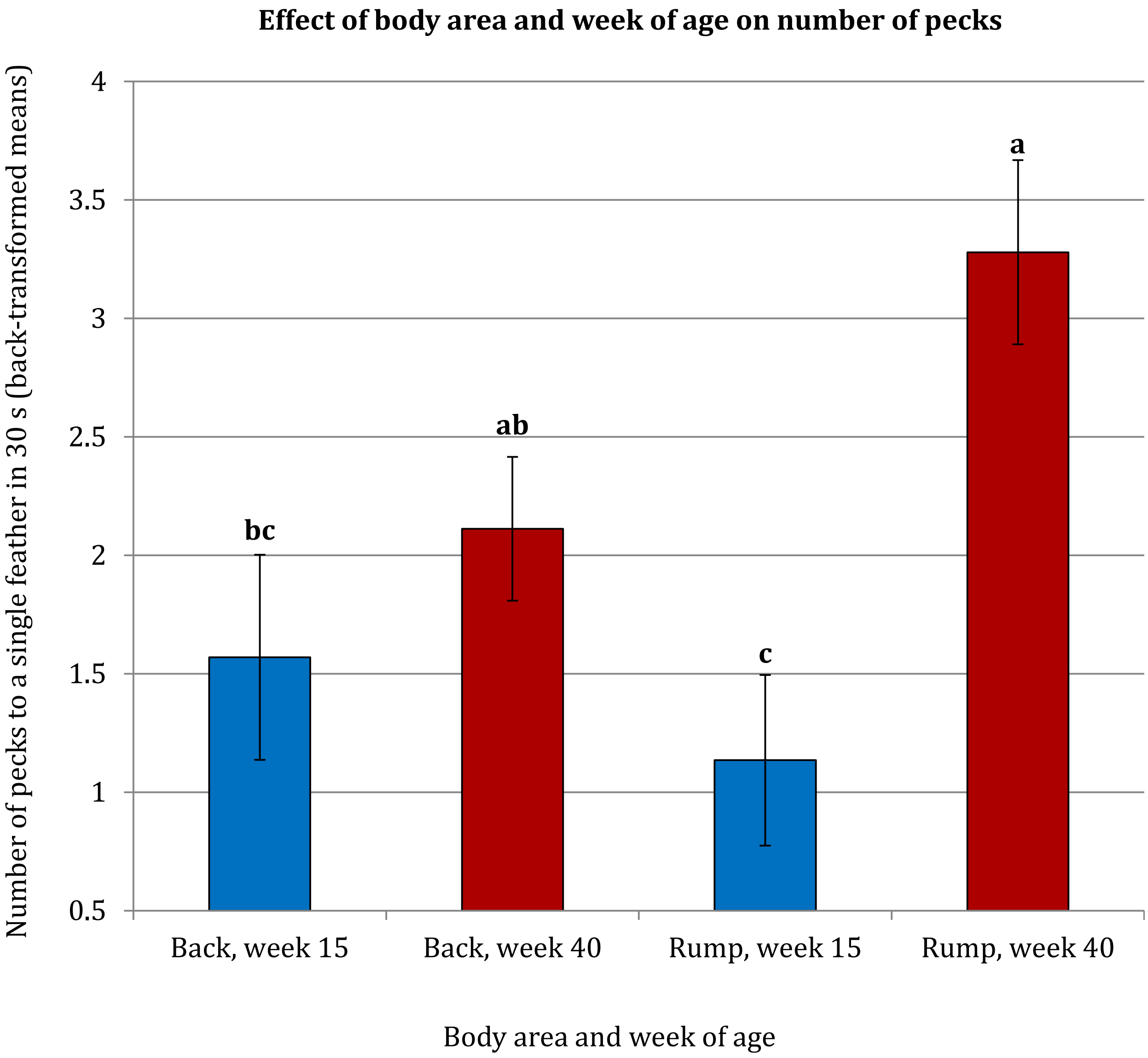

Figure 6. The effect of the body area from which feathers were sourced, and week of age at testing, on the number of pecks to a single feather presented in the home pen. Feathers were sourced from the rump or back area of a bird, and presented at 15 and 40 weeks of age. The total number of pecks per feather by all hens was analysed using the generalised linear mixed models procedure with a Poisson distribution. Columns with differing superscripts differ significantly ($P < 0.05$). Each column tends to differ from all other columns ($P < 0.1$).

DISCUSSION

Both experiments aimed to discern whether there was significant variation in plumage damage between pens of birds. Experiment 1 determined differences between pens, while experiment 2 was unsuccessful in this. It was hypothesised in experiment 1 that pens with plumage damage would have shorter latencies to peck at, and ingest single feathers presented to them, and would also be more likely to ingest feathers than pens of birds without plumage damage. An additional aim was that birds would preferentially ingest feathers from some birds over others, potentially those in the same housing facility as the tested birds. Results showed the pens with extensive plumage damage ingested more feathers, and showed shorter latencies to peck and ingest feathers. In addition, birds with plumage damage were quicker to ingest feathers taken from birds housed in the same housing facility. The results thereby support the hypothesis and suggest that birds may prefer feathers taken from particular birds. It has not been determined why feathers taken from some birds may be more attractive than others.

Experiment 2 hypothesised that birds which developed SFP would demonstrate feather-eating prior to the development of plumage damage, and that birds would preferentially ingest feathers taken from different body areas, such as the rump rather than the back area. Pens of birds that developed plumage damage did show feather-eating prior to the development of plumage damage, but the lack of variation in plumage damage between pens precluded further investigation of this relationship. Birds pecked rump feathers more than back feathers at 40 weeks of age. This result implies that birds may prefer feathers from the rump area. This should be investigated in further experiments.

Individual bird differences

All birds were reared under similar conditions, sourced from the same donor flock, and received the same diet, with the same availability of dietary fibre. The differences in plumage damage and feather-eating were observed between pens despite these environmental and genetic consistencies between pens. The differences seen between pens may therefore be attributed to individual differences between birds. Indeed, feather ingestion may have been performed by the same birds each time. The particular birds instigating SFP and feather-eating may have heightened requirements for environmental stimuli or structural elements in the diet, etc. This theory was proposed by Brunberg *et al*. (2011), who stated that SFP is performed by individual birds, regardless of environmental factors.

Age-related changes and learning

Plumage damage did not develop differentially between pens in experiment 2. Therefore, the temporal predictive relationship between plumage damage and feather-eating remains unclear. There was an effect of age, where birds were more likely to ingest feathers, and were quicker to peck and ingest feathers, at 40 compared to 15 weeks of age. It therefore appears that interest in feather-eating increased with age. Similarly, the expression of SFP and plumage damage increased with age.

De Haas *et al.* (2014b) also investigated the temporal relationship between feather-eating and plumage damage and found that flocks with severe plumage damage at 40 weeks of age tended to eat feathers more often at 15 weeks of age, compared to flocks with no severe plumage damage at 40 weeks of age. In the present experiment, feather-eating appeared to precede plumage damage and may have acted as a predictor, although since there was a lack of variability between pens this could not be fully explored. It is possible that the nutritional requirements of the birds changed with age, which affected feather-eating and SFP. Similar to the present study, McKeegan and Savory (1999) were unable to establish a causal link between SFP and feather-eating, but also commented on age-related changes, and theorised that once feather-eating is established, low availability of feathers on the pen floor can result in redirected foraging behaviour in the form of SFP.

Role of feather-eating

Birds in pens with plumage damage showed shorter latencies to peck and ingest feathers, but did not perform significantly more ground-pecking. Hence it appeared that there was a heightened interest in feathers, rather than stronger general pecking behaviour. However, the only measure of pecking motivation in the present experiment was the incidence of ground-pecking between pens. De Haas *et al.* (2010) deduced that high feather-pecking birds have a stronger pecking motivation than low feather-pecking birds, rather than a preference for eating feathers. Newberry *et al.* (2007) also found that birds which performed more SFP performed more ground-pecking during rearing, and Dixon *et al.* (2008) deduced that birds with a high foraging motivation can be more likely to perform SFP if foraging behaviour is inhibited. Kjaer (2009) suggested a hyperactivity model for SFP, whereby birds that perform more SFP have higher general activity levels.

Kriegseis *et al*. (2012) reported a decrease in SFP, and improved plumage condition, when feathers were added to the diet. Likewise, van Krimpen *et al*. (2011) stated that while the causal factors for feather-eating are unknown, feather-eating and SFP may be related to feed structure and composition. In particular, the presence of dietary fibre may minimise feather-eating. Harlander-Matauschek and Bessei (2005) suggest that feathers may be considered either as foraging material or food (Rodenburg and Koene, 2003), while Harlander-Matauschek *et al*. (2007a) suggest that besides offering a role in digestive function, feathers also represent material for exploration. Hence feather characteristics may affect their attractiveness as foraging material. Therefore, the consumption of feathers may have an exploratory basis, but nutritional influences and satiation may also play a role.

Feather characteristics

Birds preferred feathers from birds housed in the free-range facility than the cage facility or from the floor, which was demonstrated by shorter latencies to ingest the feathers from these birds. Possible characteristics affecting feather attractiveness include texture, colour, shape, olfactory properties, and gustatory stimuli provided by preen oil (Bolliger and Varga, 1961; McKeegan and Savory, 2001). Meyer *et al.* (2012) also found that feathers can alter the microbiota in the gut, which may affect bird behaviour.

Studies suggest that olfactory cues may be important in birds’ selection of feathers for eating (McKeegan and Savory, 2001), and that individual recognition may occur due to variation in composition of preen oil (Jacob and Ziswiler, 1982). Since there was variation between birds housed in separate housing facilities (cage versus the free-range facility in which the testing was conducted), the feathers may have been affected by the environment in which birds were housed, which altered birds’ speed to ingest the feathers. However, there was also a difference in the latency to ingest feathers from individual birds, so there may also be individual bird differences which affect feather properties and attractiveness for ingestion.

In experiment 2, there was a trend for an interaction effect between week of age at testing and the body area from which feathers were sourced. This result indicates that feather characteristics may vary depending on the body area from which they are taken, and that this could play a role in birds' selection of body areas for SFP and subsequent feather-eating. This was also suggested by Harlander-Matauschek *et al.* (2007b). This is in line with anecdotal observations from the present study where the rump was the first area to be targeted for SFP and exhibited the most damage. It also agrees with suggestions by McKeegan and Savory (1999) that feathers within close proximity to the uropygial gland may be particularly attractive due to their liberal supply of preen oil. The uropygial gland appears to have evolved in order to keep feathers supple and flexible with its secretion of preen oil. However, many bird species have a well-developed sense of smell, and uropygial secretions may play a role in communication between birds (Jacob and Ziswiler, 1982). Olfactory, visual, gustatory and tactile cues may be important in birds' selection of feathers for eating (McKeegan and Savory, 2001; Harlander-Matauschek and Feise, 2009; Harlander-Matauschek *et al.*, 2010). It is therefore possible that the characteristics of feathers may play a role in the selection of body areas, as well as the selection of birds which are targeted for SFP (Harlander-Matauschek *et al.*, 2007b). This should be investigated in future experiments.

Birds may have been interested in feathers despite pre-existing feathers on the pen floor due to stimulus enhancement occurring when feathers were introduced. Alternatively, the introduced feathers, having been cut from donor birds, may have possessed different and more attractive properties (olfactory, visual etc.) than the feathers on the floor, which may have been present due to a juvenile moult. Birds' responses to novelty were investigated in the novel object test.

The methodology employed in this study investigating the introduction of novel objects to the home pen, feather coverage on pen floors, and the ingestion of loose feathers prior to, and following the development of extensive plumage damage, is a method that may yield important results in future studies.

CONCLUSIONS

The results from the present study confirm the association between feather-eating and SFP. Additionally, hens showed preferences for feathers from particular hens, and from the rump area compared to the back. Pens of birds showed higher propensities for feather ingestion, as well as more plumage damage at 40 compared to 15 weeks of age. Future experiments should focus on elucidating whether feather-eating may act as a predictor of SFP.

ACKNOWLEDGEMENTS

Experiments were part of a larger project funded by the Australian Egg Corporation Limited, and a postgraduate stipend for the first author was provided by the Poultry Cooperative Research Centre, established and supported under the Australian Government’s Cooperative Research Centres Program. The authors gratefully acknowledge the assistance of Dr Evelyn Hall (University of Sydney) for her advice on statistical analyses, and Ryan Hopcroft (University of Sydney) for his help in experimental work.

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CHAPTER 7: Relationships between range access as monitored by radio frequency identification technology, fearfulness, and plumage damage in free-range laying hens

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Published in: *Animal* (2016)

10:847-53

OVERVIEW OF CHAPTER 7

The underlying behavioural trait, fearfulness, has been a key theme throughout the chapters of this thesis. Since fearfulness has been shown to play an important role in the expression of severe feather-pecking, it has featured in three of the five experimental chapters. In chapter 5, clear behavioural differences were observed between the different categories; victims and peckers. Some differences, such as the level of victimisation which was indicated by plumage damage, were obvious. The expression of severe feather-pecking was also readily visible to observers. Other behavioural differences, however, were only evident through behavioural testing. Severe feather-pecking is thought to be related to fearfulness, as discussed, but also range use in free-range systems. Although free-range systems are becoming more common in some countries, little is known about range use in free-range laying hens. The birds in chapters 3 to 6 appeared to utilise the outdoor range area extensively, but there were differing levels of plumage damage and severe feather-pecking between and within pens. Hence the experiment in chapter 7 was conducted to investigate how range use was related to both fearfulness and plumage damage. Very few studies have been conducted in this area.

ABSTRACT

Severe feather-pecking (SFP), a particularly injurious behaviour in laying hens (*Gallus gallus domesticus*), is thought to be negatively correlated with range use in free-range systems. In turn, range use is thought to be inversely associated with fearfulness, where fearful birds may be less likely to venture outside. However, very few experiments have investigated the proposed association between range use and fearfulness. This experiment investigated associations between range use (time spent outside), fearfulness, plumage damage, and body weight. Two pens of 50 ISA Brown laying hens (n = 100) were fitted with RFID transponders (contained within silicone leg rings) at 26 weeks of age. Data were then collected over 13 days. Ninety-five percent of birds accessed the outdoor run more than once per day. Birds spent an average duration of 6.1 h outside each day over 11 visits per bird per day (51.5 min per visit). The highest 15 and lowest 15 range users (n = 30), as determined by the total time spent on the range over 13 days, were selected for study. These birds were tonic immobility (TI) tested at the end of the trial and were feather-scored and weighed after TI testing. Birds with longer TI durations spent less time outside (P = 0.01). Plumage damage was not associated with range use (P = 0.68). The small group sizes used in this experiment may have been conducive to the high numbers of birds utilising the outdoor range area. The RFID technology collected a large amount of data on range access in the tagged birds, and provides a potential means for quantitatively assessing range access in laying hens. The present findings support the proposed negative association between fearfulness and range use. However, a negative association between plumage damage and range use was not supported. The relationships between range use, fearfulness, and SFP warrant further research.

Keywords: Radio Frequency Identification, range use, free-range, plumage damage, fearfulness

INTRODUCTION

Severe feather-pecking (SFP) is a highly prevalent and extremely injurious behaviour in laying hens. It causes serious negative impacts in the egg industry, in terms of both animal welfare and production efficiency. SFP is multifactorial, with contributing factors including environmental, social, genetic, and nutritional (Rodenburg *et al*., 2013). In the last half century, there has been increased intensification of animal production, including in egg production systems (Edwards, 2004). Coupled with this increase has been a recent rise in free-range housing systems in countries including Australia (Rault *et al*., 2013), and this trend is expected to continue (Richards *et al*., 2011). SFP can be a particular problem in systems in which birds are housed in large groups, as often occurs in non-cage housing systems, where the behaviour may spread via social learning (Zeltner *et al*., 2000; Rodenburg *et al*., 2013; Bessei and Kjaer, 2015).

Despite a large number of free-range farms, there is a lack of information on range use; what influences range use in individual birds, and also how use of the range may affect variables such as SFP and plumage damage. Range use is thought to be negatively correlated with rates of SFP (Lambton *et al*., 2010) and plumage damage (Green *et al*., 2000; Nicol *et al*., 2003; Mahboub *et al*., 2004). However, there are a number of factors that reportedly affect range use in laying hens, including weather, condition of the range area, pop-hole size (Hegelund *et al*., 2005; Gilani *et al*., 2014), flock size (Bestman and Wagenaar, 2003; Gebhardt-Henrich *et al*., 2014), and the presence of vertical structures (Rault *et al*., 2013), shade structures, and vegetation on the range (Bestman and Wagenaar, 2003; Zeltner and Hirt, 2003; Nicol *et al*., 2003). In particular, fearfulness may be a mitigating factor in range use, where fearful birds are thought to be less likely to access the outdoor range area (Grigor *et al*., 1995). In addition to this, fearfulness is thought to be related to SFP (Rodenburg *et al*., 2013). Birds with higher levels of fearfulness are more likely to both perform, as well as receive SFP (Rodenburg *et al*., 2004; Uitdehaag *et al*., 2009).

Fearfulness in chickens is influenced by multiple factors such as previous experience, housing conditions, interaction with humans, and genetic differences (Jones and Faure, 1981; Rodenburg *et al*., 2013). Individual variation is an important factor to consider in behavioural studies (Brunberg *et al*., 2011; Rodenburg *et al*., 2013; Bessei and Kjaer, 2015), and fearfulness is thought to vary on an individual bird level due to differences in brain morphology and serotonin turnover (Gruss and Braun, 1997; Rodenburg *et al*., 2013). While it is generally believed that there is a negative correlation between range use and fearfulness, very few studies have investigated this association, and the relationship between range use, fearfulness and SFP is relatively unexplored.

RFID technology has been used extensively in other industries, and in other species (Bonter and Bridge, 2011; Ruiz-Garcia and Lunadei, 2011; Morris *et al*., 2012). However, studies using RFID technology in domestic poultry, particularly in relation to range use, are in their infancy (Gebhardt-Henrich *et al*., 2014). The use of RFID technology has the ability to provide researchers with considerable volumes of data which were previously unattainable, and to enhance ornithological research and understandings of bird behaviour. The combination of RFID data with additional measurements such as behavioural testing and observations has the potential to increase the scope of data, and contribute to a more holistic understanding of bird behaviour (Bonter and Bridge, 2011). The present experiment investigated the association between range use, fearfulness and plumage damage. It was predicted that higher range users would be less fearful, and exhibit less plumage damage.

MATERIALS AND METHODS

Housing and husbandry

All experimental procedures were conducted in accordance with the University of Sydney Animal Ethics Committee approved protocol and with the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2004). Husbandry procedures were the same as outlined in Hartcher *et al.* (2015a), but birds were placed in the housing facility at 9 weeks of age, after being housed in the rearing facility. One hundred ISA Brown laying hens were housed in two pens of 50 birds each. Pens measured 1.83 m × 3.25 m, hence birds were kept at an indoor stocking density of approximately 8.4 birds per m^2. Wood shavings were spread over a solid concrete floor, and water and crumbled feed were available *ad libitum* via a bell-drinker (T-40 Bell Drinker, Tecnica e Innovaciones Ganaderas, S.A., Spain) and metal bell-feeder (25 kg Jumbo Feed Hopper, Protective Fabrications, Werombi, NSW, Australia), respectively. Each pen contained a five-rung timber perch unit from 13 weeks of age, each of the five perches measuring 4 cm × 125 cm. A 10-hole nest box unit was provided from 15 weeks of age. Artificial lighting was provided for 15 h per 24 h, with natural light entering the shed through pop-holes and openings at each end of the shed during daylight hours, giving an average light intensity of 52 lux across the shed.

The outdoor range areas measured 1.83 m × 10 m per pen, with 2.1 m high wire mesh fences and wire mesh across the top of all pens. The range areas were continuously accessible from 20 weeks of age via one pop-hole per pen, which measured 0.4 m high × 0.6 m wide, and allowed unimpeded access to the range area. A small winter garden measuring 2.4 m in length was present in each range area, comprising 1.2 m of metal-roofing and 1.2 m of shade cloth material. The range was covered in grass at 20 weeks of age, and subsequently denuded over the following weeks due to bird usage. No other vegetative or shade structures were present on the range. Daily weather observations were acquired from the Australian Government, Bureau of Meteorology for the suburb (Camden, NSW). Weather observations included the minimum and maximum daily temperatures (°C), average daily rainfall (mm), and average daily wind speed (km/h). Temperature and wind speed were also provided for 0900 and 1500 h daily.

Radio frequency identification technology

At 26 weeks of age, 100 birds were fitted with silicone leg rings, in which RFID transponders were contained (EVERTREND Enterprise Co., LTD Shanghai, China). The system comprised leg rings with transponders, antennas, and high-frequency readers. One antenna was placed on both sides of each pop-hole, one inside and one outside the shed, similar to the setup in Gebhardt-Henrich *et al*. (2014). Birds were required to move over both antennas in one direction, in order for the movement to be recorded by the system, thereby eliminating false movements where birds may cross one antenna but not enter or exit the shed. Antennas were wider than the pop-hole openings, to ensure that all birds were monitored upon exiting and entering the shed. The antennas enabled multiple birds to be simultaneously recorded. Antennas were dull grey in colour, 74 cm long × 33.5 cm wide × 3.5 cm high, with black cables which connected them to the readers, which were situated outside the pens.

Data on the number of visits to the range and the duration spent outside were then collected over 13 days. Leg rings were removed after 42 days. Data included a timestamp for each entry to, and exit from the shed, tag identification number, antenna number, chicken position (inside or outside), chicken action (inward or outward, determined by the sequence of antennas), outside duration, and a count of whether the bird moved from inside to outside or vice versa (0 or 1). Hens were not given time to habituate to the leg rings prior to data collection. This was because all birds were fitted with leg rings, rather than a proportion of a larger flock which was done in some other studies (Richards *et al*., 2011; Gebhardt-Henrich *et al*., 2014). Bird behaviour did not appear to be affected by the leg rings that were fitted in the present experiment. The leg rings were brown in colour, did not stand out visually to experimenters, and were constructed of a soft silicone material which was fitted against the leg of each bird. Video cameras were installed outside the shed, above each pop-hole.

To assess whether the RFID equipment interfered with hen behaviour and affected range use, data were collected on the number of birds entering the shed prior to, and following installation of the RFID system. Video footage was observed for four 30 min periods prior to the RFID system being installed, by continuously counting the number of hens entering the shed. The same observations were then conducted at the same time of day, on the second and third days after the system was installed. In order to assess whether bird behaviour was affected on the day the RFID equipment was installed, and whether birds habituated to the RFID equipment, data on range use were also collected for two 30 min periods immediately after the equipment was installed, and at the same time of day on the second and third days after the system was installed.

Of the 100 birds fitted with transponders, two distinct subpopulations were created for study. These were the highest 15 and lowest 15 (n = 30) range users, based on the total duration spent outside over the 13 days of data collection. Since the RFID technology measured when birds entered and exited the shed, rather than the extent to which they utilised the range area, this paper largely refers to range ‘access’ instead of range ‘use’, as the latter may imply how extensively birds utilise the range area. However, the two subpopulations of birds were labelled high and low range ‘users’ for convenience and readability.

Tonic immobility testing

The highest 15 and lowest 15 (n = 30) range users were subjected to a tonic immobility (TI) test at 29 weeks of age, after the conclusion of the trial. Bird RFID identification numbers were randomised, to allow the random selection of birds for the order of testing. The TI test was performed to estimate fearfulness in the selected birds, using the same method as described in Hartcher *et al*. (2015b). Birds were individually removed from the home pens, and placed on their backs with one experimenter restraining them in this position for a period of 10 s by placing one hand on the sternum and one hand over the head and eyes. Following removal of the experimenter’s hands, duration until self-righting was recorded. If the TI reaction did not persist for a minimum of 14 s, the induction procedure was repeated. The maximum number of inductions was five. The same two experimenters conducted all TI testing.

Feather-scoring

After TI testing, each bird was individually weighed and feather-scored, based on the feather-scoring procedure described in Tauson *et al*. (2005). Each bird was assigned an ordinal score of zero to three for each body area, indicating the extent of feather damage due to SFP. A score of zero denoted undamaged integument, whereas a score of three indicated extensive denuding across a body area. Body areas scored were the neck, back, sides, belly, vent, tail and rump, but the only areas with plumage damage were the rump, back, neck and tail. Following feather-scoring, the birds were marked with coloured, non-toxic paint on their backs to prevent re-testing or re-catching, and returned to their home pens.

Statistical analyses

Range use (high or low) was analysed as a factor in the fixed effect model. TI durations were not censored. Hence TI durations and body weights were analysed using a one-way Analyses of Variance. Feather-scores were analysed by ordinal regression, using the worst score per bird, out of all body areas scored. To analyse the association between continuous data sets, both the fixed effect (range use) and outcome variables (duration of TI and body weights) were treated as variates, and analysed using the Linear Mixed Models procedure. The effect of weather variables on range use were analysed using Analysis of Variance, with date in the random model. In all models, the random effect was the pen. Data were checked for normality prior to analyses with Analysis of Variance and Linear Mixed Models, and transformed if required. GenStat (15th edition, VSN International, Hemel Hempstead, UK) was used for all analyses except ordinal regression, which was performed using the ordinal library in R version 3.0.1 (R Core Team, 2015). To assess whether the RFID equipment affected range use, Analysis of Variance was used with the number of birds entering the shed as the outcome variable, the time of day as a random effect, and the day as the fixed effect, relative to when the system was installed. Two separate analyses were conducted, one using the data collected in the mornings (prior to the system installation on the first day), and one using the data collected in the afternoons (following the system installation on the first day).

RESULTS

Ninety-five percent of birds (n = 95) accessed the outdoor range more than once per day, with birds accessing the range on all days that data were collected. On average, each bird spent a total of 79.6 h outside (range 0 to 179.8 h), over 140 visits (range 0 to 780 times), for 51.5 min per visit (range 7.6 min to 3.7 h), and 6.1 h per day (range 0 to 13.8 h). When analysed as continuous variates (for n = 100 birds), there was a positive association between the total time spent outside and the average duration of visits (P = 0.02), and also between total duration outside and the number of visits (P < 0.001). Variables measured for the highest and lowest range users (n = 30) are summarised in Table 1. Low range users had longer TI durations than high range users (Table 1). There was also a negative association between TI duration and total duration outside when analysed as continuous data (P = 0.01). Birds exhibited plumage damage, as illustrated in figures 1 and 2. However, there were no associations between range use measurements and plumage condition or body weight (all $P > 0.1$). There was an effect of wind on the duration of time spent outside per day, where birds spent a longer total duration on the range when some wind was present, compared to calm days (5.55 vs 6.15 h per bird per day, P = 0.05). There were no effects of temperature or wind speed on any of the range use variables including the number of visits and the duration outside (P > 0.1). The RFID equipment did not appear to interfere with range use. There was no effect of day on the number of birds entering the shed prior to the system being installed and the two days following installation (*P* = 0.57), or after the system was installed and the subsequent two days (*P* = 0.76).

Table 1. Means and standard errors of the means for the range use, tonic immobility duration and plumage damage of free-range laying hens, categorised by their total time spent outside over 13 days.

Fixed effect	High range users	Low range users	*P* value
Total outside duration (h)	142.5 ± 5.1^{A}	16.7 ± 3.5^{B}	< 0.001
Average duration per visit (h)	$1.2 \pm 0.3^{\bullet}$	$0.5 \pm 0.1^{\bullet}$	0.06
Number of visits	233.3 ± 55.6^{A}	47.2 ± 12.2^{B}	< 0.001
Tonic immobility (s)	56.6 ± 12.6^{b}	88.9 ± 20.7^{a}	0.05
Feather-scores (ordinal score)	1.4 ± 0.2	1.3 ± 0.2	0.68

^{a,b} Values within a row with different superscripts differ significantly at *P* < 0.05.

^{A,B} Values within a row with different superscripts differ significantly at *P* < 0.001.

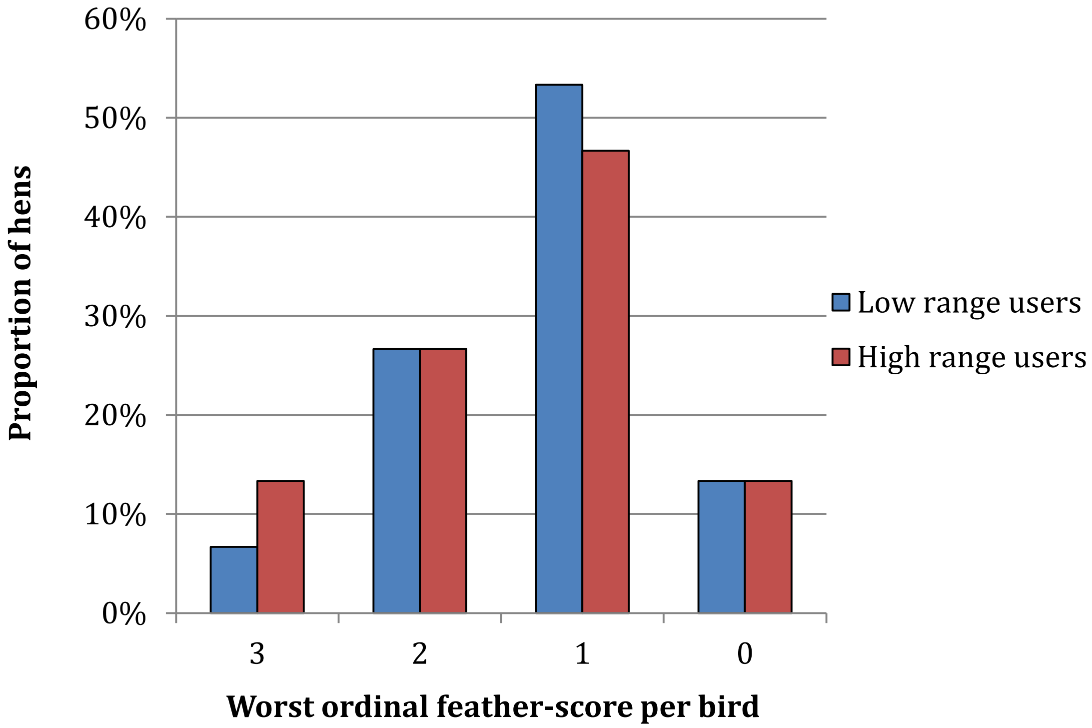

Figure 1. Proportion of hens in the high and low range use subpopulations (n = 30 hens) with each ordinal feather-score, as the worst feather-score out of all body areas per bird. A score of 3 denotes the poorest plumage condition, and a score of 0 denotes no plumage damage.

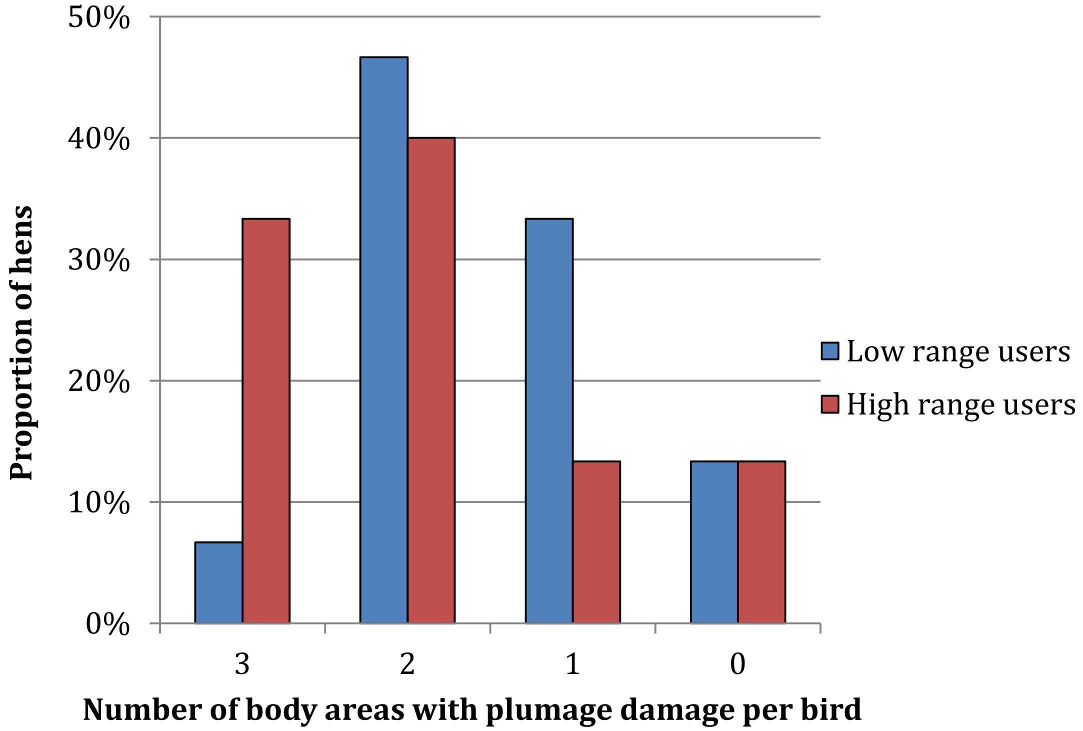

Figure 2. Proportion of hens per subpopulation (high and low range users) with each number of damaged body areas.

DISCUSSION

Range use and fearfulness

There were no differences in the numbers of birds entering the shed before the system was installed, or afterwards. Range use therefore did not appear to be affected by the placement of the antennas and the fitting of the leg rings with the RFID transponders. Birds spent more time outside on windy days, although there was no effect of wind speed. Windy days may therefore have been coupled with more cloud cover. Weather information did not include whether the day was sunny or overcast, although windy days were also on average 1°C warmer than calm days, indicating that this may have been the case. Wind may be accompanied by a higher 'wind chill factor' which may encourage range use. In total, ninety-five percent of birds accessed the range area at least once per day during the 13 days of data collection. The majority of studies in this area report much lower range use. Hegelund *et al*. (2005) studied flocks ranging from 513 to 6000 hens, and found that an average of 9% of hens used the range area, with large variations within and between flocks. Hegelund *et al*. (2006) reported that an average of 18% of hens used the outdoor range area in flocks of 1200-5000 hens, and Dawkins *et al*. (2003) found a maximum of less than 15% of the total flock accessing the range area during the day, using in-situ behaviour observations of broiler chickens on commercial farms. Bubier and Bradshaw (1998) found that three larger flocks (1500-2500 hens) had an average of 12% of hens outside during the course of the day, and the fourth flock, which comprised 490 hens, had an average of 42% hens using the outdoor range over the course of the day. The present findings support suggestions that flock size is negatively correlated with the proportion of hens that access the range (Bubier and Bradshaw, 1998; Bestman and Wagenaar, 2003; Gilani *et al*., 2014). Richards *et al*. (2011) also used RFID technology to characterise subpopulations of hens based on range use. Approximately 80% of tagged hens used the pop-holes frequently, where 10% of four flocks of 1500 hens had been tagged with transponders. There were some birds that did not appear to venture on to the range area at all, in concurrence with the data from the present study.

Longer TI durations are thought to indicate higher levels of fearfulness (Gallup Jr, 1979; Campo *et al*., 2006). Birds with longer TI durations in the present experiment spent less time outside. This finding implies a negative association between range use and fearfulness. Mahboub *et al*.

(2004) also found a negative correlation between time spent outside and TI duration. While it is generally assumed that birds with lower levels of fearfulness are more likely to venture outside, this causal relationship has yet to be confirmed. Range use may also act to decrease fearfulness. Generally, fearfulness declines with increased familiarity with the environment, and with age (Hocking *et al*., 2001). Studies have also found lower levels of fearfulness in birds housed in smaller groups (Bestman and Wagenaar, 2003; Rodenburg and Koene, 2007), and early, regular handling is thought to reduce fearfulness later in life (Jones and Faure, 1981). The birds in the present experiment were handled at least every four to six weeks throughout their lives, and people entered the pens on a daily basis to conduct husbandry procedures. The small group sizes (relative to group sizes in commercial settings), older age, and regular handling that the birds experienced may have contributed to relatively low levels of fear on a group level. This may have been a reason for the large proportion of birds which accessed the range, compared with other studies.

In addition to fearfulness, range use seems to be dependent on a number of other variables. These may include birds' previous experience with the environment and the outdoor range (Grigor *et al*., 1995; Janczak and Riber, 2015), pop-hole size, accessibility to the range (Gilani *et al*., 2014), and structures on the range (Hegelund *et al*., 2005; Rault *et al*., 2013). In the present experiment, the range areas were fenced off and had wire mesh across the top; hence the birds were protected from predators. The pop-hole in each pen was of an adequate width to allow birds to see into the range area and allow easy access to the outdoors. In addition, the small, narrow, 10 m long range areas which were protected from predators may have been conducive to high range use. All of these factors may have contributed to the high proportion of birds which utilised the range area.

Feather-scores

Birds with lower levels of fear, as assessed by TI durations, spent longer outside on the range area. However, range use was not associated with plumage damage. An association between range use and plumage damage may have been precluded in this experiment due to the very high rates of range use in the test population. The sample size was relatively small, and the study was conducted at a research facility. Results are therefore indicative of individual bird behaviour and behaviour of hens in small groups. Extrapolations to bird behaviour in commercial settings should be made with consideration of the different environmental circumstances. However, some other studies also did not find associations between plumage damage and range use. Hegelund *et al*. (2006) found no association between range use and feather-scores when researchers visited 18 organic farms periodically. Similarly, Leenstra *et al*. (2012) interviewed 257 farmers with free-range flocks, and found no relationships between feather condition and range usage. These findings suggest there may be other, sometimes overriding factors that influence range use and feather-pecking. The majority of studies, however, report an inverse association between range use and plumage damage (Bestman and Wagenaar, 2003). Mahboub *et al*. (2004) noted a negative correlation between feather damage and time spent on a grassland area outside, and Nicol *et al*. (2003) found that in a multivariate model based on farmers' reports, feather-pecking occurred at higher levels when fewer birds used the outdoor range. Green *et al*. (2000) conducted a cross-sectional survey of farmers and found similar results, where there was an association between lower range usage and feather-pecking. Lambton *et al*. (2010) visited farms to estimate range use by counting birds visible on the range at the time of the visit, and reported that increased range use may reduce SFP. Higher range use may contribute to lower plumage damage due to the higher foraging opportunities when birds access the range and the reduced stocking densities when birds are distributed across the inside and outside of the shed area.

The data from studies collected via surveys and estimations by producers yield important information relating to range use and associated factors, but should be extrapolated with care. Data collected via observations of birds accessing the range may not be able to differentiate between individual birds. Hence information has been limited in this respect, and it has not been clear whether it is the same birds that consistently access the outdoor range area or different birds on different days (Gebhardt-Henrich *et al*., 2014). The relationships between range use, fearfulness and SFP warrant further research.

Use of the radio frequency identification system

No leg rings were lost during the trial, although some numbers on the leg rings were difficult to read at the conclusion of the trial due to the build-up of manure or dirt on the leg rings obscuring the number. This is an improvement from what has been reported previously, where Freire *et al*. (2003) lost 48% of transponders which were encased in leg bands by the end of the study. Mahboub *et al*. (2004) were successful in retaining all of the transponders during their experiment, which were fixed to the wing webs rather than contained within leg bands. Thurner and Wendl (2005) also successfully used RFID technology, where transponders correctly identified 97% of laying hens. However, in the present experiment, there were a number of technological difficulties with the RFID system. These included technical problems with the functionality of the software, which limited data collection to 13 days rather than the intended 42 days. The necessitation of cables to relay information from antennas to the computer was another limitation, as only pop-holes within a close proximity (within 3 m) to a power source and computer were able to be fitted with antennas. A greater distance from the computer and longer cables could result in a loss of data. Despite technical difficulties, RFID technology proved an effective method by which to collect large amounts of data on individual birds. This was also found by Durali *et al*. (2014), who used the same RFID system, but on broiler chickens in a commercial setting.

Technologies which allow the collection and collation of large amounts of data on individual birds have the potential to play a particularly important role in future research. They may provide further substantiation for studies which have previously relied on surveys and questionnaires to estimate bird behaviour. RFID data could also be used to complement studies which utilise in-situ observations for data collection. The use of numbered leg rings in this study allowed the measurement of a number of variables which were compiled for individual birds, including plumage damage, fearfulness and body weight. Additional variables which could be recorded include in-situ behaviour observations, which could be made by marking individual birds and recording behaviour in the home pen. This would provide information on how range use relates to behaviours such as SFP, foraging, and social interactions. Further, information from RFID systems may be compiled with various measurements of biological functioning on an individual bird basis, including physiological, nutritional, as well as behavioural. In this way, future studies may provide a more holistic understanding of the behaviour, welfare, and productivity of birds housed in free-range production systems.

CONCLUSIONS

The main finding from the present experiment was that low range users had longer TI durations. This result suggests that there is a negative association between fearfulness and range use. Very few experiments have investigated this association. The RFID technology was effective in collecting large amounts of information on access to the outdoor range on an individual bird basis. Data obtained from automatic tracking systems should be combined with other measurements including physiological, nutritional, and behavioural. In this way, automatic tracking systems may be used to their full potential, and a more holistic understanding of bird behaviour, welfare and productivity may be attained.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mr Tugrul Durali (University of Sydney), who originally acquired the RFID technology for his own PhD trials, for his generous help and advice and without whom this study would not have been possible. The authors also thank Associate Professor Peter Thomson (University of Sydney) for his invaluable assistance with data consolidation methods, Dr Evelyn Hall (University of Sydney) for her advice and reassurance on methods of data analyses, and Ms Mary Tran and Mr Dean Powell for their generous assistance with practical work. This research was conducted within the Poultry CRC, established and supported under the Australian Government’s Cooperative Research Centres Program, with research funding provided by the Australian Egg Corporation Limited.

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CHAPTER 8: General Discussion

GENERAL DISCUSSION

Severe feather-pecking (SFP) is a highly injurious behaviour that is extremely prevalent in laying hens kept for commercial egg production. A number of reasons contribute to the high rate of SFP, and investigating the underlying causes is thought to be the most promising way forward in attempting to identify viable methods of control (Brunberg *et al.*, 2011). This thesis therefore investigated some potential causal factors implicated in the expression of SFP. Some experiments investigated specific aspects of husbandry and housing, while others explored the motivational basis of SFP. Primarily, this thesis aimed to understand more about the fundamental, behavioural causes of SFP, and identify some potential methods of control, and avenues for future research. Figure 1 summarises the key objectives and the main findings for each objective.

Main objectives	Key findings
Objective 1 – Investigate the effects of beak-trimming and environmental enrichment on behaviour during the rearing period	Beak-trimming and enrichment moderated behaviour during rearing. Environmental enrichment increased dustbathing and ground-scratching. Beak-trimming reduced severe feather-pecking and ground-pecking
Objective 2 – Assess the impact of beak-trimming and environmental enrichment during the rearing period on plumage damage during the laying period	Beak-trimming reduced plumage damage. Environmental enrichment reduced severe feather-pecking received, but not plumage damage
Objective 3 – Evaluate the usefulness of behavioural tests during the rearing period as predictors of plumage damage later in life	Behavioural test responses were not predictive of plumage damage
Objective 4 – Characterise some of the underlying behavioural traits in birds which perform severe feather-pecking compared to recipients of severe feather-pecking	Behavioural traits were characterised according to bird classification- peckers were more socially motivated, while recipients of severe feather-pecking were more fearful
Objective 5 – Explore the redirected foraging hypothesis	The redirected foraging hypothesis was not supported
Objective 6 – Comment on the relationship between gentle feather-pecking and severe feather-pecking	Gentle feather-pecking during rearing was not predictive of severe feather-pecking, but birds performing more severe feather-pecking during the laying period also performed more gentle feather-pecking
Objective 7 – Investigate differences between pens of birds with, and without extensive plumage damage, in their propensity for feather-eating and investigate the predictive relationship between feather-eating and severe feather-pecking	The association between feather-eating and severe feather-pecking was confirmed, a predictive relationship was not confirmed
Objective 8 – Examine the relationship between fearfulness, range use and plumage damage	An inverse relationship was found between fearfulness and range use, there was no association between plumage damage and range use

Figure 1. Diagram of the main objectives of the thesis and how each was addressed, paired with the main outcomes and conclusions.

The main objectives of the five experimental chapters comprising this thesis were as follows: 1) to investigate the effects of beak-trimming (BT) and environmental enrichment (EE) on behaviour during the rearing period; 2) to assess the impact of BT and EE during the rearing period on plumage damage during the laying period; 3) to evaluate the usefulness of behavioural tests during the rearing period as predictors of SFP later in life; 4) to characterise some of the underlying behavioural traits in adult birds which perform SFP compared to recipients of SFP; 5) to explore the hypothesis that SFP is a redirected behaviour; 6) to examine the relationship between gentle feather-pecking (GFP) and SFP; 7) to investigate differences between pens of birds with and without extensive plumage damage in their propensity for feather-eating; 8) to investigate the predictive relationship between feather-eating and SFP; and 9) to examine the relationship between fearfulness, range use and plumage damage. This discussion will explain how each of these key objectives was addressed throughout the thesis.

BEAK-TRIMMING AND ENVIRONMENTAL ENRICHMENT

BT and EE moderated pullet behaviour during the rearing period. In chapter 4, there were effects of BT and EE on bird responses in behavioural tests. Birds from enriched environments took longer to perform their initial step in the open-field test (OFT). BT birds from enriched pens performed the fewest movements in the OFT, and also took longer to ingest a food reward in the competition test than birds from other treatment combinations. These responses may imply higher levels of fearfulness in BT birds, although there were no differences in the tonic immobility (TI) test. Birds which had not been BT vocalised more, and at louder volumes than BT birds. This was interpreted as indicating that the non-BT birds had higher levels of social motivation (Forkman *et al.,* 2007). In chapter 3, EE caused clear behavioural changes. Birds in pens with EE performed more ground-scratching, and non-BT birds with no EE performed less dustbathing. These observations indicate that birds from enriched pens had greater behavioural repertoires than birds with no EE. The higher rate of ground-scratching and dustbathing was a beneficial, but unintended effect of the enrichment provided. Hens show motivation to perform dustbathing (Wichman and Keeling, 2008), and dustbathing acts to reduce feather lipids and improve the insulation capacity of the plumage (Olsson and Keeling, 2005). Similarly, hens have shown motivation to perform ground-scratching, and show signs of frustration when these behaviours are inhibited (Cooper and Albentosa, 2003). Means to improve the behavioural repertoires of birds in commercial production systems is an important consideration, and the impacts on bird behaviour in the short-term as well as the longer-term should be taken into account when providing environmental enrichment.

BT birds performed less ground-pecking and less SFP, but more GFP in chapter 3. Some of these behavioural modifications were attributed to a decreased functionality of the beaks due to BT. Freire *et al.* (2011) stated that BT removes the tip of the top and bottom mandibles, thereby reducing mechanoreception and magnetoreception in the beak. Similarly, Gentle *et al.* (1997) found that the re-grown tips of the beaks of trimmed birds contained no afferent nerves, or sensory corpuscles, unlike untrimmed controls. Freire *et al.*, 2011 proposed that increased levels of GFP are compensatory, due to the reduced sensory feedback that can occur due to BT. Reduced sensory feedback may therefore require more GFP to obtain satisfactory sensory feedback from the interaction. Gentle *et al.* (1997) came to a similar conclusion, that mild to moderate BT which results in beak regeneration, affects behaviour due to sensory deprivation due to the loss of sensory receptors in the tip of the beak. Behaviours related to feeding, such as ground-pecking, may be less affected by positive feedback, since they have more survival value. This may help to explain the results found in this thesis, where an increased rate of GFP and a decreased rate of ground-pecking were concurrently observed.

GFP plays a role in social relationships and social exploration in chicks (Riedstra and Groothuis, 2002). BT may have therefore had a negative effect on the ability of pullets to effectively perform aspects of social behaviour. However, the two types of GFP, stereotypic and social, were not differentiated in this experiment. Therefore, it is also possible the reduced exploratory behaviour, ground-pecking, caused an increase in stereotypic GFP (Blokhuis and Van Der Haar, 1989, Lambton *et al.*, 2010), which presents a negative impact on bird welfare (Lambton *et al.*, 2010, Rodenburg *et al.*, 2013).

GFP is performed using soft pecks, without grasping or pulling feathers. Conversely, SFP is performed vigorously and forcefully, and often involves the grasping and pulling of feathers (Savory, 1995). Ground-pecking has been found to have similar motor patterns to SFP (Dixon *et al.*, 2008), and can involve manipulating and picking up objects from the ground. BT appeared to compromise the effectiveness of birds' beaks to perform ground-pecking and SFP, due to the removal of the tips of the mandibles, used to grasp. The reduced ability for birds to perform ground-pecking and SFP and the lack of positive feedback from the experience may have acted as a disincentive for birds to perform these behaviours, thereby leading to reduced levels of both behaviours. Hence BT appeared to have had a positive effect by reducing the occurrence of SFP, but also negative consequences on behaviour by reducing the levels of ground-pecking (and therefore foraging behaviour) and increasing GFP.

In chapter 3, there was a reductive effect of BT, but not EE, on plumage damage at 43 weeks of age. However, in chapter 5, EE tended to reduce the amount of SFP received during the laying period. In addition, the birds which had received EE performed their first step in the OFT more quickly, indicating a lower level of fearfulness. The lack of an effect of EE during rearing on plumage damage later in life contrasts with other studies, some of which are summarised in chapter 2. However, there are many influential factors which affect the expression of SFP, and EE that elicits a particular behavioural response in one circumstance may not be effective in another, similar setting. Further, while environmental stimuli have important impacts on behaviour, individual bird variation may sometimes be overriding.

Studies investigating different types of EE are important in determining which forms of enrichment are effective in stimulating exploratory behaviour in laying hens, and reducing the expression of SFP. This could yield an improved understanding of laying hen behaviour, as well as minimising SFP. Research is required to investigate options for EE in commercial settings, to determine forms of EE which enhance the behavioural repertoire of birds and minimise the expression of SFP.

BEHAVIOURAL TESTS

Fearfulness plays an important role in the expression of SFP (de Haas *et al.*, 2010, Rodenburg *et al.*, 2013). It has been suggested that fearful birds are more likely to develop SFP (Rodenburg *et al.*, 2004a), and also that SFP causes increased fearfulness in birds which are victimised (Hughes and Duncan, 1972). While Rodenburg *et al.* (2013) identified the roles of fear and stress as one of the approaches providing the most scope for a sustainable solution for SFP, the relationship between fearfulness and SFP is not well understood (de Haas *et al.*, 2010). The directional relationship has not been determined, and it has yet to be established whether the 'victims' or the birds performing the severe pecks are more fearful (Jones *et al.*, 1995). Hence fearfulness was a key theme in three of the five experimental chapters of this thesis.

In chapter 4, behavioural tests performed during the rearing period were not statistically predictive of plumage damage later in life, based on ordinal regression analysis. There were, however, significant differences between pens in the extent of plumage damage that occurred. It can therefore be concluded that there was no relationship between fearfulness during the rearing period and plumage damage in the laying period. In chapter 5, birds with the most plumage damage had the longest durations of TI, indicating higher levels of fearfulness. When combined, the results from chapters 4 and 5 support the hypothesis that SFP causes increased fearfulness in victims, and reject the hypothesis that fearfulness predicts SFP.

As outlined in chapter 1, the ability to identify feather-peckers by phenotypic, behavioural traits could be utilised in genetic selection programs (Albentosa *et al.*, 2003). However, selection traits have not been standardised. It has been unclear whether individual birds may be categorised into distinct behavioural types, and whether there are certain behavioural characteristics associated with the propensity for individuals to perform SFP (Albentosa *et al.*, 2003, Daigle *et al.*, 2015). In chapter 5, three distinct behavioural categories were created, based on birds' involvement in SFP. Categories comprised birds which: 1) received more SFP than their conspecifics, assessed by plumage damage, 2) performed more SFP than their conspecifics, determined by *in situ* observations, or 3) were not involved in, or affected by, SFP either way. There were significant differences in the associated behavioural traits fearfulness and sociality between the three categories, and *in situ* behaviour observations and feather-scoring confirmed the differences between categories in terms of original classifications – peckers performed the most SFP, victims had the most plumage damage, while controls performed the least SFP and exhibited no plumage damage.

In chapter 5, peckers made more vocalisations and escape attempts in the OFT than victims. In chapter 4, non-BT focal birds made more vocalisations in the OFT at 5 weeks of age, and in chapter 3 they exhibited more SFP between 3 and 14 weeks of age. There are different interpretations of behaviour in the OFT, which is reflective of both coping style in response to fear-inducing stimuli, as well as the need for social reinstatement. Generally, more vocalisations and escape attempts in an OFT are interpreted as higher levels of social motivation, or a higher need for social reinstatement (Jones *et al.*, 1995, Nicol *et al.*, 2001, Forkman *et al.*, 2007). Hence, the findings from chapters 4 and 5 suggest that social factors play a role in the expression of SFP.

THE RE-DIRECTED FORAGING HYPOTHESIS

In chapter 3, ground-pecking was the only behaviour observed during rearing which was significantly related to plumage damage during the laying period. The non-BT birds performed more ground-pecking and SFP during rearing, and exhibited more plumage damage during the laying period, which was indicative of higher rates of SFP in these pens. This finding suggests that ground-pecking may be predictive of SFP and plumage damage later in life. In chapter 5, there were no associations between SFP and ground-pecking during the laying period. Therefore, the redirected foraging hypothesis was not supported by the results from chapters 3 and 5.

An inverse association between SFP and ground-pecking has been well-documented on a group level (Blokhuis and Arkes, 1984, Blokhuis, 1986, Huber-Eicher and Wechsler, 1997, Dixon *et al.*, 2010), which suggests that SFP may be ‘redirected’ foraging behaviour, or ground-pecking. However, some studies have suggested a positive association (Bilčík and Keeling, 2000, Newberry *et al.*, 2007, Rodenburg *et al.*, 2008), where birds with higher general activity and pecking motivations perform more SFP as well as more ground-pecking. The findings from chapters 3 and 5 do not support the theory that ground-pecking and SFP are inversely correlated. Rather, the results from chapter 3 agree with studies reporting a positive, or predictive relationship (Bilčík and Keeling, 2000, Newberry *et al.*, 2007), and chapter 5 concurs with those that found a lack of a relationship between ground-pecking and SFP (Blokhuis and Van Der Haar, 1989). The association between ground-pecking and plumage damage in chapter 3 was between two different life phases, suggesting a linear relationship between ground-pecking at a young age and plumage damage at an older age. Newberry *et al.* (2007) also found that birds exhibiting more foraging as chicks were more likely to develop SFP in adulthood.

GENTLE FEATHER-PECKING

Chapter 3 found no relationship between GFP during rearing and plumage damage later in life. Conversely, the BT birds which performed more GFP during rearing had better plumage condition than non-BT birds at 43 weeks of age. This suggests that GFP during rearing is not predictive of SFP in the laying period. Additionally, BT birds performed more GFP and less SFP than non-BT birds during the rearing period, suggesting an inverse relationship between the two forms of FP, although levels of SFP were low in chapter 3.

Some have theorised that GFP may develop into SFP (McAdie and Keeling, 2002, Chow and Hogan, 2005), but most studies have found no evidence of this (Rodenburg *et al.*, 2004b, Newberry *et al.*, 2007, Lambton *et al.*, 2010). In chapter 5, birds identified as peckers performed more SFP than victims and controls, and also tended to perform more GFP. Similarly, pens with higher rates of SFP also performed significantly more GFP than pens with low rates of SFP. The inverse relationship found in chapter 3 may have been influenced by BT, as well as the younger age of the birds. As theorised in chapter 3, BT may have increased GFP due to compromised sensory feedback in the beak. In chapter 5, there were no confounding effects of BT, as only the non-BT birds were studied. Therefore, while GFP and SFP are distinct behaviours, with different underlying neural mechanisms and motivations (Hughes and Buitenhuis, 2010, Rodenburg *et al.*, 2013), the results reported in chapter 5 suggest a positive association between GFP and SFP, whereby birds that perform more SFP may also perform more GFP (McAdie and Keeling, 2002, Chow and Hogan, 2005).

As outlined, GFP is thought to play a role in social exploration in young chicks (McAdie and Keeling, 2000). Results from chapter 5 suggest that there may not only be a relationship between GFP and SFP, but also a relationship between social motivation and SFP, as discussed. Understanding the underlying motivations for GFP and SFP may allow an improved understanding of why SFP occurs, and the ability to characterise phenotypic traits associated with SFP.

FEATHER-EATING

Chapter 6 investigated differences in the propensity for feather ingestion between pens of birds exhibiting extensive plumage damage, compared to birds in pens with little plumage damage. There were no effects of treatments which had previously been applied; hence the experiments were focused solely on the association between SFP and feather-eating. This chapter confirmed a link between feather-eating and SFP, in support of previous studies (Bessei *et al.*, 1999, Harlander-Matauschek *et al.*, 2010, Kjaer and Bessei, 2013, Bessei and Kjaer, 2015). Investigation of the predictive relationship between feather-eating and SFP was precluded by a lack of differentiation between pens in their development of plumage damage.

The results from both experiments in chapter 6 suggest that the properties of feathers appear to be a factor in determining which body areas and which birds are targeted for SFP. Harlander-Matauschek *et al.* (2007) suggested that feather characteristics may play a role in the selection of body areas for SFP, while others suggested that the selection of body areas for the targeting of SFP is due to the location on the birds' body, and ease of access (Bilčík and Keeling, 2000).

Feather-eating may be learnt (Ramadan and Von Borell, 2008). Birds in pens with plumage damage in chapter 6 may have expressed greater interest in the feathers partially due to learning to perform feather-eating associated with SFP behaviour. Alternatively, SFP birds may have been interested in the exploratory opportunity that feathers provide, or they may have expressed interest in the feathers due to the stimulus enhancement caused by experimenters introducing feathers to the pens. Feathers play a role in digestion (Kjaer and Bessei, 2013), and birds which perform SFP may have a specific appetite for feathers due to their intestinal microbiota (Kjaer and Bessei, 2013). Feathers possess stimulatory (visual, gustatory and olfactory) and nutritive (i.e. fibrous) qualities that are attractive to birds (Mckeegan and Savory, 2001, Harlander-Matauschek and Feise, 2009, Harlander-Matauschek *et al.*, 2010). Further research is required to elucidate the specific function that feather-eating plays, why feathers are attractive to birds for ingestion, and whether feather-eating may predict SFP.

RANGE USE

An inverse relationship between fearfulness, as indicated by TI durations and time spent on the range was reported in chapter 7. Chapters 4 and 5 agreed with previous studies, that birds which perform more SFP appear to have proactive coping styles in fear-inducing situations. This was evidenced in the OFT responses in chapter 4, and the OFT and TI responses in chapter 5. In chapter 5, the peckers had shorter TI latencies, indicating lower fearfulness and a more proactive coping style. In chapter 7, the birds with the shorter TI latencies, thereby indicating a more proactive coping style, may also be the birds which are more prone to performing SFP. However, behaviour observations were not conducted in chapter 7 to verify this potential connection.

There was no association between plumage damage and range use. Negative correlations between SFP and range use have been found in previous studies (Green *et al.*, 2000, Nicol *et al.*, 2003, Lambton *et al.*, 2010). This proposed relationship between range use and SFP was not supported in chapter 7, although these results may be specific to smaller groups of birds, as bird behaviour and the utilisation of range areas are influenced by flock size (Bestman and Wagenaar, 2003, Gebhardt-Henrich *et al.*, 2014, Gilani *et al.*, 2014). Ninety-five percent of birds frequently utilised the outdoor range, irrespective of plumage condition. This proportion of birds which used the range is much higher than what has been reported in the majority of other studies (Bubier and Bradshaw, 1998, Dawkins *et al.*, 2003, Hegelund *et al.*, 2005, 2006).

Very few studies have investigated the relationship between fear and range use in free-range systems. The negative association between the time spent outside on the range and fearfulness, as estimated by a TI test in chapter 7, is therefore an important finding, which contributes to the knowledge on ranging behaviour and fearfulness of laying hens in free-range systems. Similar experiments should be conducted in commercial situations to obtain information on these relationships in birds housed in larger group sizes.

INDIVIDUAL VARIATION

A main finding which emerged throughout the chapters was that individual variation between birds appeared to play a large and sometimes apparently overriding role in the expression of SFP. There may be a subpopulation of birds which possess genetic predispositions for SFP, or 'primary feather-peckers' (Bessei and Kjaer, 2015). These birds are thought to comprise a small proportion of the population (Jensen *et al.*, 2005). Predisposed birds may have heightened stimulatory or nutritional requirements. Bessei and Kjaer (2015) suggested that primary feather-peckers may differ in their intestinal microbiota and have a specific preference for feathers and fibre. If their particular requirements are not met, they may then instigate SFP, which can then spread throughout a group of birds via social learning (Zeltner *et al.*, 2000, Bessei and Kjaer, 2015). Therefore, environmental factors, individual bird variation and social transmission are all important in the development of SFP behaviour on a group level (all chapters, Zeltner *et al.*, 2000).

In the first two flocks which are described in all experimental chapters of this thesis, plumage damage developed differentially between pens, despite identical diets and housing conditions (when adjusting for treatment effects in some instances). A potential explanation for the disparity in the development of SFP between pens in these experiments was the unequal distribution of predisposed birds throughout the shed. The pens which developed plumage damage may have contained some primary feather-peckers, which instigated the behaviour. SFP then spread within affected pens. Conversely, pens which did not develop extensive damage to the plumage and integument perhaps did not contain individuals with predispositions for SFP.

In chapter 4, no statistical correlations were found between responses in the behaviour tests during rearing, and plumage damage in adulthood, despite extensive damage occurring in some pens and not others. These findings are contrary to some previous studies which reported correlations between responses in behavioural tests performed during rearing, and SFP in the laying period (Rodenburg *et al.*, 2004a). As outlined, this lack of a relationship may have been due to the fact that fearfulness occurs as a response to SFP, rather than acting as a precursor of SFP. The lack of a relationship may also be due, in part, to the small proportion of focal birds studied in each group. In chapters 3 and 4, four focal birds were selected at random at 11 days of age per pen of 50 birds. These focal birds were subjected to behavioural testing and *in situ* behaviour observations. Subsequently, they were feather-scored in the laying period at 43 weeks of age. This methodology allowed treatment effects to be measured, as well as the association between behaviour in rearing and plumage damage later in life. However, the focal birds represented only 8% of the population. Hence focal birds may have been selected which were not predisposed to develop SFP, and were not those which instigated the behaviour.

In the fifth chapter, focal birds were selected in adulthood. Experimenters were therefore able to select birds based on their involvement in SFP. While this resulted in a relatively small sample size, the methodology allowed direct comparisons between SFP behaviour and underlying behavioural characteristics, fearfulness and sociality. Further, 70 focal birds were selected from the eight pens in the experiment, thereby comprising a much larger proportion of each pen than in chapter 4. Chapter 5 was effective in collecting information on individual birds, and there were clear behavioural differences between victims, peckers and controls. The differences may have been due to a combination of learnt behaviours as well as the selection of some primary feather-peckers, which were predisposed to perform SFP.

When tested for their interest in loose feathers in chapter 6, birds in pens with extensive plumage damage were quicker to peck at, and ingest loose feathers. This could be because the primary feather-peckers, with a higher propensity to peck at and ingest feathers, were those that interacted with the feathers. Birds which are predisposed to perform SFP may do so due to a need for more stimulation. Alternatively, these birds may be more reactive to environmental stimuli, or have a lower threshold to respond to stimuli, which is why they interacted more quickly with loose feathers introduced into the pens. Theoretically, primary feather-peckers were not present in the pens that did not show signs of plumage damage due to SFP, and hens in these pens were thus slower to interact with feathers.

The information provided from these experiments contributes to the current understanding of which birds are involved in SFP and why it may occur. It can be difficult to track birds throughout their lives due to the time-consuming nature of behaviour observations and difficulty in collecting data on an individual bird basis, particularly in commercial systems. Therefore, information on an individual bird basis is important. Individual bird variation and genetic selection have been highlighted as potential avenues to move forward in finding preventatives for SFP (Kjaer and Bessei, 2013, Bessei and Kjaer, 2015). Future research should focus on selecting against birds with predispositions for SFP.

CONCLUSIONS

BT significantly reduced plumage damage. EE did not affect plumage damage, but may have provided some welfare benefits including an enhanced behavioural repertoire during rearing, and lower levels of SFP and fearfulness later in life. EE and BT had some effects on pullet behaviour in the OFT and competition test early in life, but there were no clear relationships between test responses in rearing, and plumage damage later in life. Chapter 5 found that peckers were more socially motivated, and less fearful, than victims of SFP. SFP appeared to cause fearfulness in recipients, rather than fear acting as a precursor for SFP.

Results from this thesis indicate that there are clear underlying behavioural differences between birds that perform SFP and those which are victimised by SFP. This appears to be influenced by social motivation, and interest in feather ingestion. Individual variation clearly plays an important role in SFP. Indeed, the two flocks utilised in all experimental chapters developed SFP differentially between the pens in the shed, irrespective of treatment. The theory that there may be particular birds within a flock that are predisposed to instigate SFP, primary feather peckers, is an important aspect to consider in future studies.

There are many reasons why some birds are more likely to perform SFP, including feeding behaviour, dietary requirements, and social motivation. The way forward in addressing the problem of SFP is first to understand it and the part it plays in the behavioural repertoire, how it is influenced by the environment, and how feather-eating may be implicated. It is important to identify the traits and requirements which contribute to particular birds initiating SFP. This may then allow the housing environment to be manipulated to address the specific requirements of these birds, and lower the risk of SFP.

Identifying specific behavioural traits in predisposed birds may also allow genetic selection against the phenotypic traits associated with the likelihood to perform SFP. Genetic selection studies should be paired with, or complemented by, studies investigating environmental effects. This has the potential to result in birds with lower predispositions to perform SFP, housed in environments which effectively cater for their behavioural needs, thereby reducing the risk of the development of SFP.

The findings from this thesis suggest that individual bird differences are crucial when aiming to investigate the causes of SFP. Efforts should be made to:

1) Understand why some birds are predisposed to perform SFP and what their specific requirements may be – whether they may have different dietary requirements, heightened requirements for environmental stimuli, or lower thresholds and greater reactivity to environmental stimuli.

2) Determine whether the particular phenotypic traits associated with the expression of SFP may be consistently characterised and selected against in breeding programs.

3) Elucidate the role feather-eating plays in the expression of SFP and whether feather-eating may predict SFP.

4) Identify environmental modifications that may be made to the housing to attempt to meet birds' biological needs, as well as effectively abate SFP.

In conclusion, while the environment and providing appropriate stimuli are important for bird welfare, attempts to control SFP should acknowledge the importance of individual bird differences. Some birds appear to have particular predispositions, which must be addressed when aiming to understand and control SFP, rather than solely attempting to control SFP on a group level. While housing and husbandry methods are made commercially at a group level, it is clear that they may not be effective in addressing and controlling SFP, and consolidated efforts to understand SFP on an individual bird level is of fundamental importance. Future research should focus on selecting against individuals with predispositions for SFP. In the shorter term, efforts should be made to minimise fearfulness, as well as investigate bird responses to different forms of EE, whilst utilising the BT procedure to mitigate the effects of SFP in the meantime.

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