Measures of behavioural reactivity and their relationships to carcass and meat quality in sheep

by

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Abstract

The ability to measure behaviour and knowledge of the relationships between temperament, stress and productivity in livestock can be utilised in improving livestock production systems, minimising stress and maximising ease of handling and efficiency of production. This research aimed to further the understanding of behavioural reactivity in sheep and investigate links between reactivity, carcass composition and meat quality. This was achieved with a combination of experimental trials and interviews of livestock transporters.

Evidence to support the concept of temperament and behavioural reactivity was gathered across the studies. Repeatable differences between sheep were demonstrated, with moderate to strong correlations between some behavioural tests and links between reactivity and physiological indices of stress. Heritability estimates of up to 0.20 were reported; combined with significant breed effects on reactivity this provides evidence of an inherent genetic component of behavioural reactivity.

Sheep experience was rated as very important by livestock transporters. Age and experience, although confounded, also appeared to be important in the experimental trials. Older, more experienced lambs were less reactive and their behaviour more repeatable than when measured at a younger age.

Although all of the behaviours investigated contributed to overall reactivity, restrained and unrestrained tests are only weakly correlated, indicating that these tests measure distinctly different components of behaviour. A consistent finding in the literature review and experimental chapters was greater reactivity in ewes compared to wethers, although livestock transporters indicated that sex was of minimal

importance and ewes and wethers were behaviourally indistinguishable when handled as a mob.

Few phenotypic or genetic relationships were found between the behaviours and carcass traits in initial analysis of an industry research flock dataset. However, in an experimental trial behavioural reactivity was related to carcass quality, albeit opposite to the relationship expected, with higher reactivity being associated with better loin pH. Lambs that were more reactive at behavioural testing appeared to be stressed in lairage, most likely as they were moved to the stunning area, triggering lactic acid production, resulting in lower loin pH 24 hours post slaughter.

Methodological advances were made during this research. The first of these was in regards to the measurement of flight speed, validating this behavioural test in sheep and assessing the appropriate distance for use in this species. This thesis also assessed the usefulness of face cover score and hairline position as indicators for a variety of measures of behavioural reactivity. The results give strong evidence against the future use of facial hair patterning as an indicator for behaviour in this species.

These results show that behavioural reactivity on farm, combining flight speed and restrained tests and measured later in life (after weaning), can be used to predict the reaction of the sheep at slaughter. The complex phenomenon of reactivity was successfully divided into its components, significantly advancing the understanding of behaviour in sheep. Further work is necessary to confirm these results in a variety of flocks and to establish the links between individual behavioural tests and stress. Greater understanding of the relationships between the behavioural and physiological responses to stress will improve both farm productivity and animal welfare.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works.

Dodd, C.L., Pitchford, W.S., Hocking Edwards, J.E. and Hazel, S.J. 2012., Measures of behavioural reactivity and their relationships with production traits in sheep: a review. *Applied Animal Behaviour Science,* vol. 140, pp.1-15.

Dodd, C.L., Hocking Edwards, J.E., Hazel, S.J. and Pitchford, W.S., 2013. Genetic and non-genetic effects on flight speed and agitation in weaned lambs. *Association for the Advancement of Animal Breeding and Genetics*, Napier, New Zealand, vol. 20, pp. 114-117.

Dodd, C.L., Hocking Edwards, J.E., Hazel, S.J. and Pitchford, W.S., 2014. Flight speed and agitation in weaned lambs: Genetic and non-genetic effects and relationships with carcass quality. *Livestock Science*, vol. 160, pp. 12-20.

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23rd June 2014

Author contributions

This thesis contains three peer-reviewed articles (Chapters 2, 4 and 5) and one manuscript submitted for publication (Chapter 3). Chapters 6 to 9 have been prepared for publication but not yet submitted. All of these articles and manuscripts are multi-authored but I am the lead author on each. Descriptions of the involvement of each author and their agreement to the inclusion of the manuscript in this thesis are provided in the authorship statement at the start of each chapter. A brief overview of the involvement of the authors in each article is provided below.

Associate Professor Wayne Pitchford, Dr Susan Hazel and Dr Janelle Hocking Edwards provided advice and editorial assistance for all manuscripts. Associate Professor Wayne Pitchford provided statistical advice for Chapters 3-8. Dr Susan Hazel provided assistance in qualitative analysis for Chapter 9.

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Manuscripts included as chapters

The following publications have arisen from research conducted during PhD candidature and are included in the thesis as individual chapters. Contributions of co-authors are described in authorship statements that appear prior to each article.

Published

Chapter 2. Literature review

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Chapter 4. Flight speed and agitation in the Information Nucleus Flock

Dodd, C.L., Hocking Edwards, J.E., Hazel, S.J. and Pitchford, W.S., 2013. Genetic and non-genetic effects on flight speed and agitation in weaned lambs. *Association for the Advancement of Animal Breeding and Genetics*, Napier, New Zealand, vol. 20, pp. 114-117.

Chapter 5. Relationships of flight speed and agitation with carcass and meat quality in the Information Nucleus Flock

Dodd, C.L., Hocking Edwards, J.E., Hazel, S.J. and Pitchford, W.S., 2014. Flight speed and agitation in weaned lambs: Genetic and non-genetic effects and relationships with carcass quality. *Livestock Science*, vol. 160, pp. 12-20.

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Chapter 3. Optimising the measurement of flight speed in sheep

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The Information Nucleus flock dataset formed a significant resource in the preparation of this thesis. The Information Nucleus and associated research programs are supported by the Australian Government's Cooperative Research Centres Program, Meat and Livestock Australia and Australian Wool Innovation Ltd. I gratefully acknowledge the contributions of the many research staff involved with the Information Nucleus program, as well as the generous support provided to the program by Australian sheep breeders. This program has been a very large collaborative effort involving teams of scientists and technical officers from 7 different research agencies working at 9 Information Nucleus flock sites, 7 abattoirs and 7 laboratories across Australia. Individual people are not listed simply because of the number involved, but their contributions are duly acknowledged. Flock management and data collection have been an essential part of this study that would not have occurred without the dedication and efforts of these people.

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Chapter 1. Introduction

Stock handling training at The University of Adelaide, South Australia, October 2011.

1.1 Context

Stress is the response of the body to any demand made on it in an effort to return to homeostasis (Selye, 1973; Moberg, 2000). Stressors can be positive or negative, and either psychological, such as from restraint, handling or novelty, or physical, from hunger, thirst, fatigue, injury or thermal extremes (Moberg, 2000). Welfare exists on a continuum whereby in a state of good welfare an animal can adapt to stressors in its environment to maintain homeostasis with minimal effort and in a state of poor welfare the animal fails to adapt and suffers (Veissier and Boissy, 2007). In addition, it is not the way in which these stressors affect the animal, but how the animal perceives them that determines the animal's state of welfare. Factors that influence how an animal perceives stressors include novelty, pleasantness, controllability and the opportunity for the animal to satisfy its needs; using these factors the animal forms an emotional response to its situation (Veissier and Boissy, 2007).

Temperament in domesticated livestock is most often defined as an individual's behavioural and physiological response to stressors such as handling by humans (Burrow, 1997). Specifically, temperament is an animal's inherent potential to respond in a particular way to a stimulus. Temperament does not change over time, but its expression is influenced by the animal's environment (Bates *et al.*, 1995). The phenotypic expression of temperament is behavioural reactivity, and this can change over time.

Behavioural reactivity is influenced by a number of factors including sex, genotype, and experience and it has been demonstrated that it is possible to create divergent populations by selection on behavioural traits (Beausoleil *et al.*, 2008; Amdi *et al.*, 2010). Positive experiences during handling can improve the response of sheep to human interactions, reducing fear behaviours, but this effect disappears after negative experiences (Hutson, 1985; Hargreaves and Hutson, 1990b). Studies have shown that cattle handled in a positive way, with minimal negative human-animal interactions, have increased productivity (Hemsworth *et al.*, 2000; Hemsworth *et al.*, 2002; Hanna *et al.*, 2006), but equivalent work has not been conducted in sheep.

A range of tests have been developed and trialled to assess different aspects of sheep behaviour when exposed to stressful conditions. No single test is likely to be sufficient to identify all the behaviours which producers aim to improve. However, there remains value in each type of test in assessing specific facets of sheep reactivity and temperament. Behavioural tests can be broadly classified into restrained tests, where animals are physically restricted in a box or weigh crate, and non-restrained tests, conducted in larger areas such as paddocks or arenas. Behavioural tests can be objective or subjective and vary in their repeatability and heritability, although estimates of heritability and repeatability have not been made for many behavioural tests. In many cases it is also unclear how the behaviours exhibited in these tests relate to stress in the sheep.

It is also important to understand the relationships between behavioural reactivity and productivity in sheep. To date several studies in sheep have investigated the link between behavioural reactivity and weight gain, generally showing that calmer lambs in restrained tests had higher average daily gains than did nervous sheep (Pajor *et al.*, 2007; Pajor *et al.*, 2008; Pajor *et al.*, 2010; Horton and Miller, 2011) although this has been contradicted in non-restrained tests (Wolf *et al.*, 2008; Amdi *et al.*, 2010). There are no known studies that have investigated the effects of behavioural reactivity on carcass and meat quality traits in sheep, although some work has been done in cattle indicating significant links between reactivity and bruising (Fordyce *et al.*, 1985; Fordyce *et al.*, 1988).

1.2 Aims and objectives

The initial focus for this research was to assess a range of measures of behavioural reactivity to identify one or more that were suitable for use in large research or commercial contexts. Such a measure should be easily incorporated into normal management procedures, and be repeatable and heritable, with known relationships to stress.

This research also aimed to assess the effect of quantity of handling on sheep behavioural reactivity. Several studies have been done in the past which have measured behavioural reactivity in research flocks subject to more frequent handling than would be experienced by commercial flocks of sheep. It is important to understand how this extra handling may have affected the results of these behavioural studies in order to determine whether these research sheep are representative of commercial sheep in their reactivity. The impacts of other variables, such as sex, breed, previous experience and facilities, on behaviour of sheep was also investigated. From the literature, it was hypothesised that ewes would be more reactive than wethers and that there would be differences in reactivity between breeds.

In order to establish the importance of behavioural reactivity, its relationship to profitability needed to be assessed. This primarily involved investigating links between reactivity, carcass composition and meat quality. It was expected that associations would be seen between behaviours in sheep on farm and carcass traits such as meat pH, with less reactive lambs exhibiting higher quality carcasses (i.e. lower loin pH).

1.3 Research approach

Three main components formed the research for this thesis. A dataset containing observations of 11,047 lambs, including behavioural reactivity scores (measured at weaning), weights, parasite resistance scores, carcass composition and meat quality measurements was available for analysis. These lambs were born in 2008-2010 and had pedigree recorded for up to three generations. This dataset was analysed using a linear mixed model to investigate the relationships between the two behaviours (flight speed and agitation), heritability of behaviours and relationships with carcass composition and meat quality. The results of this analysis are presented in Chapters 4 and 5.

Two experimental trials were also conducted. The first of these involved 251 lambs, which were split into two groups to test the impact of frequency of handling. One group was handled at approximately the same frequency as would be common for commercial sheep, i.e. only for necessary management procedures, and the second was handled more frequently to simulate the extra handling often received by research flocks. Both groups underwent behavioural testing seven days after weaning (average age 104 days), at post-weaning (average age 240 days) and within three weeks of slaughter (average age 279 days), to test the repeatability of behavioural measures and effect of age on behavioural reactivity. Saliva samples were collected at each testing point to investigate the relationship between the behaviours tested and stress. A variety of behavioural tests were used in order to compare their repeatability and ease of measurement. These lambs were then slaughtered, and carcass composition, meat quality and serum stress metabolites measured, for assessment of links between reactivity and carcass traits. The results of this trial are presented in Chapters 6, 7 and 8.

The second experimental trial aimed to assess the most appropriate distance over which to measure flight speed. This trial utilised 48 lambs and assessed five distances, repeated over two days. The effects of management group, sex and day of testing were also evaluated. The results of this trial are presented in Chapter 3.

Finally, qualitative research interviews of livestock transporters were used to explore the spectrum of attitudes and experiences surrounding ease of handling of sheep in yards. This section was designed to achieve a ‘big picture’ overview of the factors affecting sheep behaviour in an industry context, to improve understanding of how people perceived the relationships between the factors affecting ease of handling. The results of this qualitative study are presented in Chapter 9.

1.4 Relevance of thesis

The main contribution of this thesis has been to improve understanding of temperament and behavioural reactivity in sheep, the relationships between these and productivity, and their importance as perceived by livestock transporters. This thesis is unique in investigating many different behavioural tests in sheep at once, and in examining relationships with carcass composition and quality. The use of livestock transporter experience as a resource is also novel.

Chapter 2. Literature review

The article presented in this chapter reviews the use of behavioural testing in sheep, including the types of tests used, factors that affect reactivity and the relationships between reactivity and productivity. This article was published in Applied Animal Behaviour Science in 2012, volume 140, pages 1-15.

Sheep near Angaston, South Australia, April 2011.

Authorship Statement

Cathy Burnard was responsible for critical review of the literature, writing the manuscript and was corresponding author.

23rd June 2014

Signature Date

Wayne Pitchford supervised the development of this work and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

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NOTE:
This publication is included on pages 11-25 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

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Chapter 3. Optimising the measurement of flight speed in sheep

The article in Chapter 3 examines the protocol for measurement of flight speed in sheep. Estimates of repeatability found during review of the literature were lower and more variable than would be expected given the success of the measure in cattle, where the test was developed. This study tests the hypothesis that a distance shorter than 1.7m would be more appropriate because sheep are smaller than cattle. This article was submitted for publication in *Applied Animal Behaviour Science* in May 2014.

Sheep being flight speed tested at Roseworthy Campus, The University of Adelaide, South Australia, August 2013.

Authorship Statement

Cathy Burnard was responsible for data collection and analysis, writing the manuscript and was corresponding author.

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Signature Date

Wayne Pitchford supervised the development of this work and assisted with statistical analysis and in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

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Abstract

Flight speed was developed as a measure of cattle behaviour in the late 1980s. Subsequent studies in sheep used the 1.7m distance specified in the original cattle methods, despite a lack of evidence optimising the measure for sheep. The aim of this study was to investigate the repeatability and variance of flight speed measured at a range of distances from 0.5m to 2.0m in order to recommend the most suitable distance for measurement of flight speed in sheep, with the hypothesis that a distance shorter than 1.7m would be more appropriate because sheep are smaller than cattle.

Flight speed was repeatable across days (R^2 = 0.88-0.97) and within days (R^2 = 0.54-0.66). Residual correlations between speeds measured at the different distances were very high (>0.82), suggesting that while greater distances are better to maximise variance, shorter distances will still be adequate where necessary. The residual variance was consistent across distances but the between sheep variance increased with distance at which flight speed was measured so within the range of distances assessed, the greater distance is preferable. Ability to detect differences between sheep based on sex, pen and day of measurement increased with distance of measurement. Pen differences in flight speed were significant at distances of 1.0m and greater, and ewe lambs were faster than wethers when measured at a distance of at least 1.3m. Lambs were faster on the first day than the second day when measured at 1.7 or 2.0m. These results indicate that flight speed is better measured over longer distances up to 2.0m, is highly repeatable in young lambs and phenotypic variation exists with which to discriminate between animals.

3.1 Introduction

The ability to measure behaviour allows researchers to investigate the relationships between temperament, stress and productivity in livestock. High reactivity is associated with lower productivity in livestock species, and relationships between flight speed and average daily gain, feed conversion efficiency, body condition, carcass dressing percentage and meat tenderness in cattle exist (Petherick et al., 2002; Kadel et al., 2006; Müller and von Keyserlingk, 2006; Petherick et al., 2009b). If animals can be discriminated based on behavioural reactivity, the amount of stress experienced during routine handling procedures may be reduced either genetically (by selective use of animals with lower reactivity for breeding) or non-genetically (e.g. by modification of behaviour through training programs or modification of handling techniques for particularly susceptible animals). Flight speed was developed as a measure of cattle behaviour in the late 1980s, after it was observed that agitated cattle exit the weigh crate more rapidly than their calmer counterparts (Burrow et al., 1988). The test has subsequently been used in sheep. Previous studies (Pajor et al., 2008; Hocking Edwards et al., 2011; Plush et al., 2011; Dodd et al., 2014) in sheep have used the 1.7m distance specified in the methods of the original paper describing flight speed in cattle (Burrow et al., 1988). The 1.7m distance in cattle was selected for practical reasons, being slightly less than the length of a standard cattle crush, and comparative studies of other distances are lacking. None of these previous studies attempted to determine the repeatability of flight speed in sheep.

Two studies in sheep differ from the original cattle literature in their methodology. Due to practical constraints, one study used distances of 1.0m and 2.0m measured concurrently (Horton et al., 2009). They found high correlations between speeds to each distance (r = 0.86-0.92) and a high degree of consistency (r = 0.46-0.65) between repeated measures within a day regardless of distance (Horton et al., 2009). In contrast, in another study which also used distances of 1.0m and 2.0m flight speed was not at all repeatable ($r < 0.01$) (Blache and Ferguson, 2005). The present study aimed to determine if the distance over which flight speed is measured is important for the variance and repeatability of flight speed.

3.2 Methods

3.2.1 Animals and measurements

This trial utilised 48, 10-14 week old Merino x Border Leicester lambs housed in four 700m^2 pens each containing 12 lambs. Each pen contained equal numbers of ewe and wether lambs. Animal use was approved by the Animal Ethics Committees of the University of Adelaide and Primary Industries and Resources of South Australia.

The trial was conducted over two days, one week apart. Although temperatures were similar on the two days (maximums 14 and 17°C), day 1 was clear and sunny while day 2 was overcast and raining. Temporary yards were erected outdoors in a laneway between the home paddocks of the lambs. Lambs were handled by five different handlers, randomised across pens and repeats such that all pens of sheep were handled by at least two different handlers. On each day, lambs were moved one pen at a time to a small holding yard attached to a sheep crate. The handler then moved lambs one at a time in random order into the crate, where their ear tag number was read and recorded. The front gate of the weigh crate was then unlatched and opened by the handler, allowing the lamb to exit the crate via a route defined by two open-sided fences set parallel to each other forming a 2m long raceway. A striped scale showing 1cm delineations was attached to one fence and the flight of the lamb past this scale was video recorded. The end of the raceway opened into the existing laneway between paddocks and the lamb was allowed to return to its paddock of origin before the next lamb was placed in the crate to avoid influencing the flight speed of the next lamb. This was completed with all four pens of lambs before repeating in random order, such that all four pens were handled twice on Day 1. All pens were measured again on Day 2, but due to rain, only one pen was repeated. In total, three pens were handled three times and one pen was handled four times.

Video footage of lamb movement past the scale was broken down into frames and the position on the scale of the lamb at each frame was recorded. Frames were 0.02 seconds apart. Based on the number of frames, the times taken for lambs to travel 0.5m, 1.0m, 1.3m, 1.7m and 2.0m were calculated and then converted to speeds (m/s) to each distance.

3.2.2 Statistical analysis

Analyses of flight speeds were conducted in ASReml version 3 (Gilmour et al., 2009) using the univariate model below:

$FS_{ijklmn} = \mu + S_i + P_j + D_k + L_l + D_k x L_l + H_m + e_{ijklmn}$

where FS_{ijklmn} is the flight speed at a given distance, for lamb (L) l (1-48), of sex (S) i (ewe, wether), from pen (P) j (1-4), measured on day (D) k (1,2), handled by handler (H) m (1-5), with a residual or error of e_{ijklmn}, where S, P and D are fixed effects and L, DxL and H are random. This model was used to test significance for each of the fixed effects, in addition to the proportion of variance attributable to handlers (H/(L + DxL + H + e)), and repeatability within ((L + DxL)/(L + DxL +e)) and across days (L/(L + DxL) of flight speed (Falconer, 1981). A significance threshold of $P<0.05$ was used for the fixed effects.

A multivariate model with fixed effects of sex, pen and day and random effects of sheep x day and handler was fitted to flight speeds at all five distances simultaneously to obtain residual correlations between flight speed measured at different distances for a sheep on a given day for a given run.

3.3 Results

The variance in flight speed increased with distance with both increases and decreases in speed observed (Figure 3.1). Between sheep variance both within and across days increased with distance, while residual and handler variance remained static (Figure 3.2). Overall there was a 27% increase in total variance across the distances measured (Table 3.1). Differences in handlers accounted for less than 5% of the variance in flight speed.

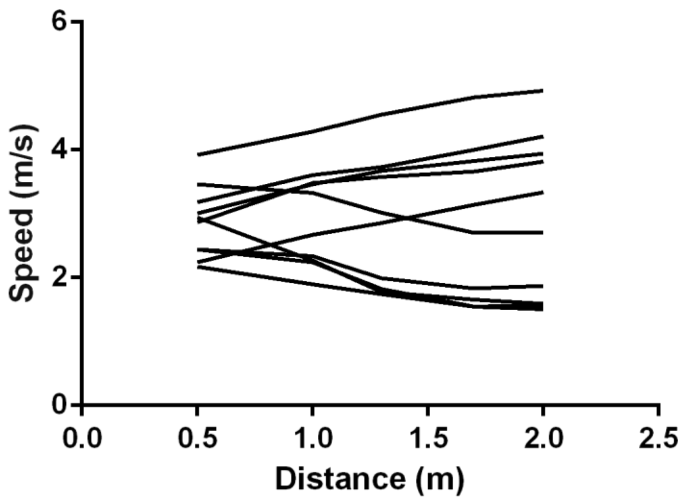

Figure 3.1. Example divergence of sheep flight speeds over five distances from 0.5m to 2.0m.

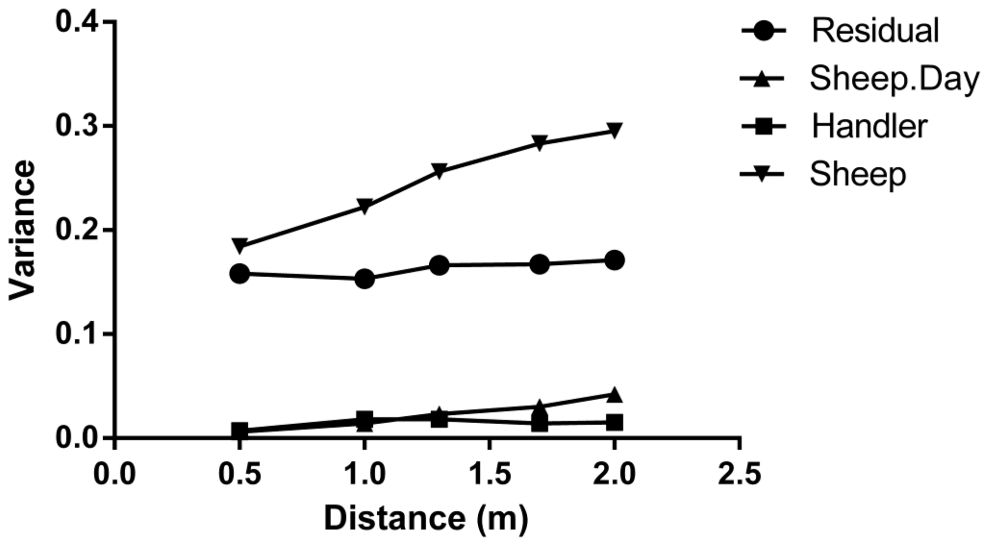

Figure 3.2. Variance components of flight speed in sheep over five distances from 0.5m to 2.0m.

Repeatability both within a day (technical repeatability) and across days (biological repeatability) was very high (Table 3.1). Flight speeds were highly correlated across distances, with all correlations between consecutive distances >0.92 and a correlation between flight speed at 0.5m and 2.0m of 0.82.

Table 3.1. Repeatability (R^2) within and across days for flight speed measured over five distances from 0.5m to 2.0m, and residual correlations between flight speed measured at different distances for a sheep on a given day.

Distance	0.5m	1.0m	1.3m	1.7m	2.0m
Model 1 single distance					
Total variance (m^2/s^2)	0.36	0.41	0.46	0.50	0.52
R^2 within Day	0.54	0.61	0.63	0.65	0.66
R^2 across Day	0.97	0.94	0.92	0.90	0.88
Model 2 multi-distance					
0.5m		0.92	0.88	0.85	0.82
1.0m			0.98	0.95	0.93
1.3m				0.98	0.96
1.7m					0.99

A significant effect of pen occurred at distances of 1.0m and greater, with differences in flight speed of 26-29% between pens (Table 3.2). At distances of 1.3m and above, ewe lambs were 12-14% faster (3.2-3.3m/s ±0.14) than wether lambs (2.8-2.9m/s ±1.4). Lambs were slightly faster on the initial day of testing (3.2m/s ±0.11) compared to the second day one week later (2.9-3.0m/s ±0.12) over 1.7m and 2.0m distances.

Table 3.2. Tests of significance (F-probabilities) of fixed effects on flight speeds over five distances from 0.5m to 2.0m.

Distance	0.5m	1.0m	1.3m	1.7m	2.0m
Sex	0.141	0.053	0.049*	0.032*	0.025*
Pen	0.062	0.024*	0.018*	0.023*	0.026*
Day	0.152	0.110	0.053	0.031*	0.024*

* = significant at $P<0.05$

3.4 Discussion

The purpose of this study was to investigate the repeatability and variance of flight speed measured at a range of distances from 0.5m to 2.0m in order to recommend the most appropriate distance for measurement in sheep. Flight speed was highly repeatable, a result supported by previous findings in sheep (Horton et al., 2009). The low values reported by Blache and Ferguson (2005) were likely a result of their protocol, with flight speed measured upon exit from a solid sided box after a period of visual isolation from flock mates. In contrast, exit from a weight crate where animals are restrained, but are not completely enclosed is standard practice for both cattle and sheep. The between sheep variance increased but the residual was relatively constant across the distance at which flight speed was measured, while both technical (within day) and biological (across day) repeatability remained high. This indicates that, within the range of distances assessed, the greater distances are preferable. As biological repeatability was very high, suggesting that lambs were unlikely to re-rank over the period of time tested here (one week), multiple measurements on a single day would be sufficient to estimate flight speed in lambs. Greater distances have greater power to detect treatment differences, with an increase in between sheep variance from 52% at 0.5m to 56% at 2.0m. However, correlations between distances were very high, indicating that shorter distances will still be adequate where necessary, as suggested previously (Horton et al., 2009). Further work needs to be done to establish that this is also true in older sheep.

Variance, particularly between sheep variance, increased with the distance of measurement (Figure 3.1). Additionally, at longer distances the ability of the test to detect differences between lambs based on pen, day of measurement and sex also increased. There were differences between days for flight speed measured at the distances of 1.7m and 2.0, with lambs travelling faster on the first day compared to the second. Given the high repeatability of flight speed, this day effect would not have a large impact on ranking of sheep in experiments with measurements on a single day. The difference between days is likely due to habituation to the testing protocol, and has been reported previously. Flight speed in cattle was also observed to decrease with successive measurements (King et al., 2006), and reductions in reactivity and fear behaviours are seen with repeated arena testing in sheep (Kilgour and Szantar-Coddington, 1995; Kilgour, 1998; Erhard et al., 2006; Wolf et al., 2008). As lambs gain experience of handling, their behavioural responses adapt to their perception of the situation. Differences in the rate of this adaptation could have important consequences for the consistency of relative ranking of individuals over time.

At distances of 1.3m and greater, ewe lambs had faster flight speeds than wether lambs. This sex effect has been reported many times, with previous studies concluding that females are more fearful of restraint, humans, novelty, surprise and isolation than males (Le Neindre et al., 1993; Vandenheede and Bouissou, 1993; Viérin and Bouissou, 2003; Boissy et al., 2005; Dodd et al., 2014). While it has been suggested that females are more socially motivated than males, greater cortisol responses to both stress and ACTH administration indicated that there are also functional differences in the hypothalamic-pituitary-adrenal axis between sexes (Turner et al., 2002; van Lier et al., 2003).

In conclusion, the results of this study indicate that flight speed is best measured at distances of 1.7-2.0m, although it is impossible to say based on this data whether distances greater than 2.0m may be even better. These distances maximise repeatability of the measure and show the greatest degree of between sheep variance, improving the ability of the test to detect differences between lambs.

Chapter 4. Flight speed and agitation in the Information Nucleus Flock

The Cooperative Research Centre for Sheep Industry Innovation, supported by the Australian Government's Cooperative Research Centres Program, Meat and Livestock Australia, Australian Wool Innovation Ltd and state-based primary industries research agencies, developed the Information Nucleus flocks for use in sheep industry research. The sheep in these flocks have known pedigree and are phenotyped for an extensive array of traits, including behavioural reactivity in the form of agitation and flight speed. The first two years of data from this resource had been analysed previously to estimate heritability of the behavioural traits; this chapter reports the updated results including the three years data available. This paper was presented by Cathy Burnard at the Association for the Advancement of Animal Breeding and Genetics conference in Napier, New Zealand in October 2013.

Michael Aldridge, Sam Walkom, Cathy Burnard and Wayne Pitchford at Te Mata peak, New Zealand, October 2013.

Authorship Statement

Cathy Burnard was responsible for data analysis, writing the manuscript and was corresponding author.

23rd June 2014

Signature Date

Wayne Pitchford supervised the development of this work and assisted with statistical analysis and in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Susan Hazel supervised the development of this work and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

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Signature Date

Janelle Hocking Edwards supervised the development of this work and assisted in data collection and editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

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Dodd, C.L., Hocking Edwards, J.E., Hazel, S.J. & Pitchford, W.S. (2013). Genetic and non-genetic effects on flight speed and agitation in weaned lambs. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics 20th Conference, v. 20, pp.114-117*

NOTE:
This publication is included on pages 41 - 44 in the print copy of the thesis held in the University of Adelaide Library.

Chapter 5. Relationships of flight speed and agitation with carcass and meat quality in the Information Nucleus Flock

Chapter 5 present the results of the further analysis of the Information Nucleus data, incorporating carcass phenotypes to examine the relationships of flight speed and agitation with carcass composition and meat quality. This article was published in *Livestock Science* in 2014, volume 160, pages 12-20.

Information Nucleus progeny being moved at Kybybolite Research Centre, South Australia, December 2011.

Authorship Statement

Cathy Burnard was responsible for data analysis, writing the manuscript and was corresponding author.

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Signature Date

Wayne Pitchford supervised the development of this work and assisted with statistical analysis and in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

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Dodd, C.L., Hocking Edwards, J.E., Hazel, S.J. & Pitchford, W.S. (2014). Flight speed and agitation in weaned lambs: Genetic and non-genetic effects and relationships with carcass quality.
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It is also available online to authorised users at:

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Chapter 6. Test type and age of testing affect assessment of behavioural reactivity in lambs

Investigations of the Information Nucleus dataset failed to show strong links between behavioural reactivity and carcass traits. Behaviours in the Information Nucleus were only tested at weaning, and only two behaviours were used. The following trials aimed to find out if weaning really is the best time to measure reactivity in sheep and if the type of test used affects the conclusion regarding temperament of the individual sheep. This chapter has been prepared as a manuscript for submission to *Applied Animal Behaviour Science*. Some of these results were presented by Cathy Burnard at the 64th Annual Meeting of the European Federation of Animal Science in Nantes, France in August 2013.

Sheep at Struan Research Centre during behavioural testing, April 2012.

Authorship Statement

Cathy Burnard was responsible for data collection and analysis, writing the manuscript and was corresponding author.

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Signature Date

Wayne Pitchford supervised the development of this work and assisted with statistical analysis and in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

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Janelle Hocking Edwards supervised the development of this work and data collection and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

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Signature Date

Abstract

Behavioural reactivity is the phenotypic expression of temperament, an animal's inherent potential to respond in a particular way to a stressful stimulus. High behavioural reactivity in sheep can affect productivity, directly via decreased growth rates, or indirectly through increased labour in handling and injuries to both handlers and animals. A variety of behavioural tests have been trialled in sheep in an effort to quantify behavioural reactivity and assess its impact on productivity. This study aimed to explore the relationships between various measures of behaviour in lambs, and assess the impact of a number of factors including frequency of handling, age of measurement and sex on behavioural reactivity.

This trial utilised 251 lambs, born between 15 July and 13 September 2011 to Merino and Border Leicester x Merino (BLM) dams by Poll Dorset sires. Seven days after weaning, lambs were randomly assigned to treatment groups receiving high or low frequency of handling. Behavioural measures were conducted on lambs seven days after weaning (average age 104 days), at post-weaning (average age 240 days) and within three weeks of slaughter (average age 279 days). Behavioural measures included agitation while visually isolated, a subjective crate score, coefficient of variation of weight, objective and subjective flight speeds and order of entrance of animals into the weigh crate. Salivary cortisol concentration and heart rate were used as physiological indicators of stress.

Principal components analysis yielded three components explaining 54% of the variance in lamb behaviour. Component one (PC1, 29% of variance) had positive loadings for all behaviours, regardless of testing context, so was interpreted as

temperament. Component two (PC2, 14% of variance) had positive loadings for restrained tests (agitation, subjective crate score and CV of weight) and negative loadings for objective and subjective flight speeds, and was interpreted as expressing context-specific behavioural reactivity. Component three (PC3, 11% of variance) had primarily positive loadings for measures at weaning (0.08-0.54) and primarily negative loadings for post-weaning and pre-slaughter measures (-0.57-0.19), indicating the impact on behavioural reactivity of measurement age, likely due to sheep experience.

Ewe lambs had higher values for PC3 than wether lambs. Breed had a significant effect on PC1, with lambs from crossbred dams displaying lower values than lambs from purebred Merino dams. There were no significant relationships between the principal components describing lamb behaviour and salivary cortisol, handling treatment or heart rate.

Temperament can be illustrated using a combination of restrained and non-restrained tests. These different types of tests measure different aspects of temperament, likely representing differences in the perception of the stressful stimulus by the sheep. The timing of measurement affects behavioural reactivity, with measures at weaning differing from those conducted later in life. Further work is necessary to determine the relationships between reactivity and stress in lambs, and to continue these measures in older sheep.

6.1 Introduction

Behavioural reactivity of sheep can impact on many aspects of husbandry. Sheep that are more reactive to routine handling procedures can be harder to handle resulting in wasted time (Hutson, 1985), and injuries to both handlers and animals. Additionally a sheep's reactivity can affect production traits such as growth (Pajor *et al.*, 2007; Pajor *et al.*, 2008; Pajor *et al.*, 2010; Horton and Miller, 2011) and maternal ability (Murphy *et al.*, 1994; Gelez *et al.*, 2003; Plush *et al.*, 2011), and thus has economic importance to livestock industries.

Behavioural reactivity is the phenotypic expression of temperament, an animal's inherent potential to respond in a particular way to a stressful stimulus. Temperament is defined by genetics and permanent environmental effects such as early life experience; behavioural reactivity is also influenced by an animal's temporary environment (Bates *et al.*, 1995) and physiological state (Viérin and Bouissou, 2001). There are known effects of sex and breed on reactivity in sheep, with females demonstrating greater reactivity to isolation (Boissy *et al.*, 2005), and differences in coping strategies reported between different breeds (Le Neindre *et al.*, 1993). Additionally, there appears to be additive genetic effects on reactivity; parents selected based on their reactivity reliably produce offspring of similar temperament, and divergent parents produce offspring of intermediate reactivity (Le Neindre *et al.*, 1993; Beausoleil *et al.*, 2008; Amdi *et al.*, 2010). Environmental influences such as previous experience (Romeyer and Bouissou, 1992; Viérin and Bouissou, 2003; Erhard *et al.*, 2006) and health status (Fell *et al.*, 1991; Adams and Fell, 1997) affect the way an animal perceives the immediate stimulus, and thus affect the behavioural reactivity of sheep. Underlying all of these factors is the latent variable "inherent temperament". Behavioural reactivity is the measured phenotype reflecting how the animal's temperament affects its reaction to a given stimulus or test.

A variety of behavioural tests have been trialled in sheep in an effort to quantify behavioural reactivity and assess its impact on productivity. Agitation uses an electronic agitation meter to record vibrations generated by movement and vocalisation of the lamb while it is isolated visually from flock mates (Blache and Ferguson, 2005). Studies evaluating the relationships between agitation and maternal ability have concluded that agitation is unlikely to be linked to lamb survival (Hocking Edwards *et al.*, 2011; Plush *et al.*, 2011) although links with other production traits have not yet been considered. Initial assessments during the development of this test indicate very high repeatability (0.76) over intervals of two days between testing, making agitation a promising candidate for further investigation (Blache and Ferguson, 2005).

The subjective crate score (Pajor *et al.*, 2007; Pajor *et al.*, 2008; Pajor *et al.*, 2010) uses a one to five score to describe the movement of a lamb in the weigh crate. This test also appears to be highly repeatable, with estimates of 0.55-0.71 over a 49 day interval (Pajor *et al.*, 2008). Coefficient of variation (CV) of weight measures the degree of movement of a lamb within a weigh crate using fluctuations of its weight, similar to the crate score, and has reported repeatability estimates of greater than 0.44 (Horton and Miller, 2011). Both the subjective crate score and CV of weight have demonstrated relationships with growth indicating a link to productivity (Pajor *et al.*, 2008; Horton and Miller, 2011).

Other behavioural tests, such as exit speed from a weight crate (flight speed), have been successfully used in cattle with demonstrated links to growth rate and cortisol (Fell *et al.*, 1999; Müller and von Keyserlingk, 2006), and a high degree of within-animal consistency (Müller and von Keyserlingk, 2006). Subjective flight speed, which classifies the gait of the animal as it exits the weigh crate, may be particularly useful in a commercial setting due to its simplicity and ease of application (Hoppe *et al.*, 2010). Similarly, the order of entrance of animals into the weigh crate is a simple-to-record trait which has been successfully linked to behavioural reactivity in cattle (Hoppe *et al.*, 2010).

It is important to understand the relationship of behavioural reactivity to stress in order to accurately interpret behavioural patterns and to potentially utilise behavioural reactivity as an informative tool in improving livestock production systems and minimising stress to the sheep. Salivary cortisol quantification is a minimally invasive method, and provides an indicator of stress in sheep. Rises in salivary cortisol correspond to the introduction of stressful stimuli and are similar in pattern to changes in plasma cortisol concentration (Yates *et al.*, 2010; Matsuura *et al.*, 2012). Despite the inherent variability of hypothalamic-pituitary-adrenal axis activity between individuals, influenced by environmental factors and genetics, cortisol remains a common approach to the study of stress in livestock (Mormède *et al.*, 2007). In addition, heart rate can be measured with relatively little equipment and cost and provides another indicator of physical or psychological disturbance in sheep (Syme and Elphick, 1982; Hargreaves and Hutson, 1990a), though again with inherent variability between individuals (Baldock *et al.*, 1988).

Effects of handling frequency have been demonstrated in cattle, for example steers with a high frequency of handling were less reactive during handling (Francisco *et al*., 2012). Likewise in sheep, animals exposed to daily gentle handling were more receptive of a human than those that did not receive daily handling, although no differences were seen in response to restraint or if the daily handling was aversive (Mateo *et al*., 1991). Sheep raised in research flocks are often subjected to handling with greater frequency than those in commercial flocks, potentially affecting their representativeness to sheep reactivity in commercial flocks.

This study explored the relationships between various measures of behaviour in lambs, and assessed the impact of a number of factors including frequency of handling, age of measurement and sex on behavioural reactivity. Specifically, this research evaluated whether behavioural studies conducted in the Cooperative Research Centre for Sheep Industry Innovation's "Information Nucleus" flocks could be generalised to the commercial population of sheep given the high frequency of handling that occurs in these research flocks. Salivary cortisol concentration and heart rate were used as physiological indicators of stress.

6.2 Methods

6.2.1 Animals

This trial utilised 251 lambs, born between 15 July and 13 September 2011 to Merino and Border Leicester x Merino dams by Poll Dorset sires (1st and 2nd cross respectively). These lambs were progeny of sheep bred within the Cooperative

Research Centre for Sheep Industry Innovation's "Information Nucleus" flock at Struan Research Centre, South Australia (van der Werf *et al.*, 2010). Lambing occurred across six paddocks and lambs were collected into a single flock at the end of lambing. The maximum difference in ages of lambs was 60 days. Lambs were weaned at 78 to 138 days of age, 11 weeks after the end of lambing. Seven days after weaning lambs were randomly assigned to High- or Low-Handling treatment groups. Groups were balanced for weaning weight, sex and dam breed. After weaning, Low-Handling lambs were yarded only for necessary management procedures, such as shearing and animal health procedures including drenching, vaccination and crutching, and for behavioural testing. High-Handling lambs were also yarded for these procedures but were additionally handled through the yards approximately every 3 weeks in order to test the effect of frequent handling on behavioural reactivity. Lambs were grown on green dryland annual pastures until weaning and then irrigated pastures (annual ryegrass/clover) until slaughter. This project was approved by the Animal Ethics Committees of the University of Adelaide and Primary Industries and Resources of South Australia.

6.2.2 Behavioural assessments

Behavioural measures were conducted on lambs seven days after weaning (average age 104 days), at post-weaning (average age 240 days) and within three weeks of slaughter (average age 279 days). Weaning and pre-slaughter behavioural measures were collected over two consecutive days and post-weaning measures were collected over three consecutive days. Behavioural measures were collected by a team of two to three people at each time point, with at least one person common to all three measurement periods. During testing external noises were minimized and no dogs were permitted near the sheep to reduce the impact of these stressors on the behaviour of the sheep.

Saliva samples were collected during the post-weaning and pre-slaughter testing periods (Yates *et al.*, 2010). Prior to the first behavioural test, the lamb was restrained and a saliva sample taken (initial cortisol) using a Salivette synthetic swab (Sarstedt, Germany). A second saliva sample was collected just prior to entry to the weigh crate (final cortisol). The time between the initial and final saliva samples ranged from 17 to 113 minutes. Swabs were stored in individual vials to prevent cross-contamination on ice for no more than two hours before freezing at -20°C.

After initial saliva sample collection, agitation of the lamb was measured by placing it in a fully enclosed isolation box with dimensions 1.5m x 0.7m x 1.5m (Blache and Ferguson, 2005). The number of vibrations caused by movement and vocalisation of the lamb over 30 seconds were measured using an agitation meter. The agitation meter was calibrated using a standard “electro-sheep” (Blache and Ferguson, 2005). After the agitation test, the lamb was released into a section of raceway for holding. Initial saliva samples and agitation testing were conducted on groups of approximately 20 lambs prior to beginning the next test on the first lamb.

During the weaning behavioural testing period, heart rates of lambs were measured while held in the raceway using a stethoscope over 15 seconds. Heart rates were not collected at the post-weaning and pre-slaughter testing periods due to difficulty locating the heart sounds of larger lambs.

After collecting the second saliva swab, each lamb entered the weigh crate. Weight and coefficient of variation (CV) of weight were recorded using Agitation v2.01 software (Horton and Miller, 2011) in conjunction with a Tru-Test XR3000 weigh unit (Tru-Test Ltd., New Zealand). The CV of weight was calculated by the software using 10 weights per second, over 20 seconds for a total of 200 weights per lamb. During this time (20 sec) a subjective crate score was assigned to the lamb, ranging from 1: Calm, no movement, to 5: Constant movements and shaking the crate (Pajor *et al.*, 2008). The lamb was then released from the weigh crate and allowed to leave without interference or prompting. Time to pass between two infra-red start and stop beams (1.7m apart) attached to a timer was recorded and flight speed was calculated (m/s) (Burrow and Dillon, 1997). A subjective score of walk, trot, run or leap was also allocated to the exit behaviour of each lamb, based on the method used in cattle (Hoppe *et al.*, 2010).

The order in which lambs came through the isolation box was recorded across each period and from this relative order was calculated as the relative percentage scaled position of each lamb such that the first lamb that was recorded in the period had the lowest relative order number and the last lamb measured in the period was assigned a relative order of 100.

6.2.3 Cortisol assay

Saliva samples were frozen and stored at -20°C on the swabs before assaying. On the day of assay, swabs were thawed and centrifuged at 1620*g* for 20 minutes.

Saliva (300µL-1000µL per Salivette) was transferred to microtubes and centrifuged at 18,626*g* for 1 minute to precipitate any solids. Cortisol levels were analysed using an ELISA test (DetectX Cortisol EIA kit, Arbor Assays, USA) validated for sheep saliva. Samples were run in triplicate. The cortisol ELISA has a sensitivity of 17.3pg/mL, and all samples tested had intra-assay coefficients of variation of less than 10%.

6.2.4 Statistical analysis

Data was analysed in R (R Core Team, 2013; Revelle, 2013) using Principal Component Analysis with a correlation matrix (Table 6.2) and no rotation. The procedure was performed using both Pearson's and Spearman's correlations; very similar results were obtained and only those using the Pearson's correlations are reported herein. Relative orders were excluded due to the lack of correlations both within these measures and between these and other variables. Summary statistics for the remaining behaviours are shown in Table 6.1. An initial analysis was run to obtain eigenvalues for each component in the data. Five components had eigenvalues over Kaiser's criterion of 1 and in combination explained 70% of the variance. The scree plot (not presented) showed inflections that would justify retaining three components. Three components explained 54% of the variance and it was considered that two extra components added little to the analysis, thus three components were retained in the final analysis (Table 6.3).

Salivary cortisol data was analysed via regression, testing the effects of initial vs final and post-weaning vs pre-slaughter on salivary cortisol concentration. Initial vs final was significant and so it was decided that an average value of cortisol was not appropriate. Final salivary cortisol adjusted for initial cortisol, both across and within measurement periods, was fitted to each principal component as described below. In separate models, initial cortisol was fitted, across and within period, to each principal component.

General linear regressions were fitted to each of the three components retained:

$PC_{ijklmnopq} = \mu + W_i + G_j + S_k + B_l + H_m + R_n + C_o + P_p\ e_{ijklmnopq}$

with fixed effects of weaning weight (W) i, average daily gain (G) from weaning to pre-slaughter j, sex (S) k (ewe or wether), breed (B) l (1^{st} or 2^{nd} cross), handling treatment (H) m (High- or Low-Handling), heart rate at weaning (R) n and salivary cortisol (C) o ($initial_{PW}$ + $final_{PW}$, $initial_{PS}$ + $final_{PS}$, $initial_{\mu}$ + $final_{\mu}$, $initial_{PW}$, $initial_{PS}$, $initial_{\mu}$). A random effect of lambing paddock (P) p (6 paddocks) was also included.

These same regressions were also fitted to three behaviours which were strongly correlated with the PCs to assist with interpretation: subjective flight speed (post-weaning) for PC1, agitation (post-weaning) for PC2 and subjective flight speed (weaning) for PC3.

Table 6.1. Summary statistics for behavioural tests at weaning, post-weaning and pre-slaughter in lambs.

	Count	Min	Max	Mean	SD
Agitation (W)	234	0	141	27.6	24.3
Agitation (PW)	176	1	151	38.0	26.4
Agitation (PS)	216	0	91	24.0	18.5
subCrate (W)	231	1	5	1.6	0.9
subCrate (PW)	176	1	5	1.6	0.8
subCrate (PS)	216	1	4	1.6	0.7
CV (W)	231	0.28	2.40	1.11	0.47
CV (PW)	175	0.28	2.31	0.84	0.35
CV (PS)	216	0.18	2.77	0.90	0.41
objFS (W)	227	0.28	3.95	1.50	0.69
objFS (PW)	168	0.42	3.40	1.39	0.66
objFS (PS)	208	0.52	3.70	2.06	0.68
subFS (W)	232	1	4	1.7	0.9
subFS (PW)	176	1	3	1.5	0.8
subFS (PS)	216	1	4	1.9	0.8

W = weaning; PW = post-weaning; PS = pre-slaughter; subCrate = crate score; CV = coefficient of variation of weight; objFS = objective flight speed; subFS = subjective flight speed.

6.3 Results

Moderate to strong correlations were seen between some behavioural tests, particularly between subjective and objective measures of the same behaviour (Table 6.2). Correlations between values for the same behavioural test over different testing periods were generally also moderate to strong. However, relative orders were not correlated either across time within the measure or with other behavioural tests.

Table 6.2. Pearson correlation (r) matrix between measures of behaviour in lambs. Values are multiplied by ten and reported to one significant figure.

	AG (W)	AG (PW)	AG (PS)	subCrate (W)	subCrate (PW)	subCrate (PS)	CV (W)	CV (PW)	CV (PS)	objFS (W)	objFS (PW)	objFS (PS)	subFS (W)	subFS (PW)	subFS (PS)	Order (W)	Order (PW)	Order (PS)	Order (S)
AG (W)		4	2	2	1	0	2	1	0	0	0	0	0	0	1	0	-1	0	0
AG (PW)	4		3	2	1	1	2	2	0	1	1	0	1	0	0	1	-4	0	0
AG (PS)	2	3		2	1	2	2	1	2	1	1	1	2	1	1	1	1	-2	0
subCrate (W)	2	2	2		0	1	5	0	2	2	1	0	3	0	1	-1	0	-1	1
subCrate (PW)	1	1	1	0		3	0	5	3	1	3	2	1	3	2	1	-1	0	0
subCrate (PS)	0	1	2	1	3		1	2	7	2	3	4	2	3	4	1	1	-1	0
CV (W)	2	2	2	5	0	1		1	1	1	0	1	1	0	1	0	0	0	0
CV (PW)	1	2	1	0	5	2	1		3	1	2	1	0	1	1	1	0	0	-1
CV (PS)	0	0	2	2	3	7	1	3		2	2	3	2	3	4	0	1	0	-1
objFS (W)	0	1	1	2	1	2	1	1	2		4	4	8	4	4	1	0	0	1
objFS (PW)	0	1	1	1	3	3	0	2	2	4		6	3	8	5	0	-2	-2	1
objFS (PS)	0	0	1	0	2	4	1	1	3	4	6		3	5	8	1	1	-1	1
subFS (W)	0	1	2	3	1	2	1	0	2	8	3	3		3	4	0	0	0	1
subFS (PW)	0	0	1	0	3	3	0	1	3	4	8	5	3		6	0	-1	-2	1
subFS (PS)	1	0	1	1	2	4	1	1	4	4	5	8	4	6		0	1	-1	1
Order (W)	0	1	1	-1	1	1	0	1	0	1	0	1	0	0	0		0	0	1
Order (PW)	-1	-4	1	0	-1	1	0	0	1	0	-2	1	0	-1	1	0		1	1
Order (PS)	0	0	-2	-1	0	-1	0	0	0	0	-2	-1	0	-2	-1	0	1		0
Order (S)	0	0	0	1	0	0	0	-1	-1	1	1	1	1	1	1	1	1	0	

W = weaning; PW = post-weaning; PS = pre-slaughter; S = slaughter; AG = agitation; subCrate = crate score; CV = coefficient of variation of weight; objFS = objective flight speed; subFS = subjective flight speed; Order = relative order.

Three components explained 54% of the variance in lamb behaviour (Table 6.3). Component one (PC1, 29% of variance) had positive loadings for all behaviours. Component two (PC2, 14% of variance) had positive loadings for restrained tests (agitation, subjective crate score and CV of weight) and negative loadings for objective and subjective flight speed (Figure 6.1). Component three (PC3, 11% of variance) had primarily positive loadings for measures at weaning (0.08-0.54) and primarily negative loadings for post-weaning and pre-slaughter measures (-0.57-0.19).

Table 6.3. Summary factor loadings for PCA analysis of lamb behavioural testing (n=251).

	PC1	PC2	PC3	PC4	PC5
Agitation (W)	0.14	0.63	0.08	0.36	-0.24
Agitation (PW)	0.19	0.61	0.08	0.45	0.00
Agitation (PS)	0.33	0.44	0.19	0.03	-0.18
subCrate (W)	0.29	0.48	0.44	-0.30	0.02
subCrate (PW)	0.43	0.20	-0.57	0.18	0.38
subCrate (PS)	0.62	0.15	-0.33	-0.44	-0.07
CV (W)	0.21	0.53	0.30	-0.19	-0.17
CV (PW)	0.34	0.35	-0.53	0.14	0.40
CV (PS)	0.59	0.20	-0.34	-0.56	-0.02
objFS (W)	0.62	-0.17	0.48	0.04	0.49
objFS (PW)	0.75	-0.26	-0.05	0.37	-0.15
objFS (PS)	0.74	-0.29	0.01	0.02	-0.35
subFS (W)	0.58	-0.14	0.54	-0.06	0.47
subFS (PW)	0.75	-0.30	-0.05	0.29	-0.14
subFS (PS)	0.78	-0.24	0.00	-0.05	-0.30
Eigenvalue	4.32	2.05	1.68	1.25	1.15
Proportion of variance	0.29	0.14	0.11	0.08	0.08

W = weaning; PW = post-weaning; PS = pre-slaughter; subCrate = crate score; CV = coefficient of variation of weight; objFS = objective flight speed; subFS = subjective flight speed.

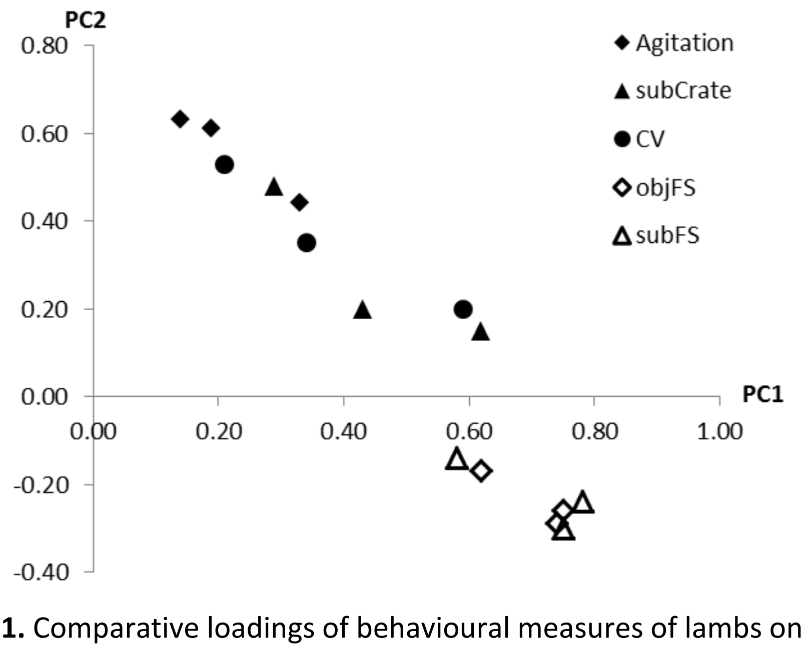


Figure 6.1. Comparative loadings of behavioural measures of lambs on principal components 1 and 2.

PC1 was related to sex, breed and initial cortisol at post-weaning, although sex was not significant at $P<0.05$. This model accounted for 14% of the variance in PC1. Wether lambs had higher scores than ewes for PC1 and its representative behaviour, subjective flight speed at post-weaning (wethers 1.66±0.09, ewes 1.35±0.08, P=0.003). Second cross lambs had lower PC1 values and slower subjective flight speeds at post-weaning (1.38±0.10) than their 1st cross counterparts (1.63±0.07, P=0.048). Initial cortisol at post-weaning was significantly related to PC1, but not post-weaning subjective flight speed, such that higher PC1 scores were associated with lower initial salivary cortisol (Table 6.4).

Although not significant at $P<0.05$, PC2 was related to sex (P=0.01) and average daily gain (P=0.07), with the model accounting for 82% of the variance in PC2. Ewe lambs scored more highly on PC2, and there was a negative association between the principal component and average daily gain. This effect of growth rate on PC2 was maintained regardless of its position in the model in respect to sex, breed and handling group. These trends with sex and average daily gain were also seen in post-weaning agitation scores (wethers 35.9±3.05, ewes 37.8±2.80), although again these were not significant (P>0.50). Initial cortisol at post-weaning was significantly related to PC2 (P=0.04) and post-weaning agitation (P=0.001) such that higher PC2 and agitation scores were associated with lower initial salivary cortisol.

Ewe lambs scored more highly on PC3 than did wether lambs (P=0.04). This effect was not seen in subjective flight speed at weaning (P=0.60). There were no other significant effects of the fixed factors or covariates on PC3. This model accounted for 14% of the variance in PC3.

There were no significant relationships between the principal components describing lamb behaviour and final salivary cortisol across or within measurement periods, or initial salivary cortisol across measurement periods. Weaning weight, handling treatment and heart rate at weaning were not related to any of the three principal components describing lamb behaviour (Table 6.4). Lambing paddock accounted for a negligible proportion of variance for PC1 and PC2, but explained approximately 6% of the total variance for PC3.

Table 6.4. Tests of significance (F-probabilities) of fixed effects on principal components describing lamb behaviour, and on individual behaviours representing these components.

	PC1	PC2	PC3	subFS(PW)	Agit(PW)	subFS(W)
Weaning weight	0.92	0.41	0.23	0.82	0.02*	0.19
ADG	0.14	0.07†	0.42	0.18	0.25	0.66
Sex	0.08†	0.10†	0.04*	0.003*	0.53	0.60
Breed	0.03*	0.88	0.14	0.05*	0.62	0.35
Handling	0.67	0.19	0.98	1.00	0.23	0.68
Heart rate	0.99	0.56	0.96	0.62	0.49	0.39
Init Cortisol (PW)	0.01*	0.04*	0.56	0.30	0.001*	0.02*

ADG = average daily gain; Init = initial; W = weaning; PW = post-weaning; subFS = subjective flight speed; Agit = agitation. †P<0.10, * P<0.05.

6.4 Discussion

This study aimed to explore the relationships between various measures of behaviour in lambs, and assessed the impact of a number of factors including frequency of handling, age of measurement and sex on behavioural reactivity. Correlations between consecutive measurements of behaviour were generally moderate to strong (0.30-0.57), indicating reasonable repeatability across time for these measures. The most striking exception to this was relative order which had a maximum correlation across time of r=0.14 and correlations between consecutive measures of 0.02-0.06 (Table 6.2). Relative order was not a repeatable measure and is likely to be of limited value in sheep, and thus was not included in further analysis.

Principal component analysis of five behaviours across three times yielded three components which together explained 54% of the variance in lamb behaviour. PC1 had positive loadings for all of the behaviours, and was interpreted as temperament. Loadings on this component ranged from 0.14 (agitation at weaning) to 0.78 (subjective flight speed pre-slaughter). Behavioural reactivity is the reaction of an animal to a specific stimulus, and is a characteristic of the individual sheep, influenced by sex, breed, genetics and environment (Le Neindre *et al.*, 1993; Adams and Fell, 1997; Boissy *et al.*, 2005; Erhard *et al.*, 2006; Beausoleil *et al.*, 2008; Amdi *et al.*, 2010). Underlying these is the animal's inherent temperament which transcends individual tests; this is the trait that appears to be expressed in PC1.

The second component had opposite loadings for flight speed (objective and subjective) and restrained measures (agitation, CV of weight and subjective crate score), supporting conclusions from previous work that these different types of tests measure different aspects of temperament (Blache and Ferguson, 2005; Hocking Edwards *et al.*, 2011; Plush *et al.*, 2011). Anecdotally, some sheep are known to 'freeze' when restrained, giving low agitation and crate scores, and then bolt when release, resulting in fast flight speeds. This may be due to the different contexts of the behavioural reactions resulting in differences in the perception of the stressful stimulus by the sheep; while restrained tests measure the reaction to isolation from conspecifics, the other behavioural tests herein may quantify reaction to human handling. That these tests measure different aspects of temperament may be an asset when assessing links between reactivity and productivity. A range of tests which describe behaviour in different contexts will provide greater opportunities to accurately evaluate relationships with production traits. Temperament is an animal's inherent potential to respond in a particular way to a stressful stimulus. The expression of this potential (behavioural reactivity) is influenced by, among other things, context. That PC2 differentiates between tests in different contexts indicates that the PC may represent the behavioural reactivity of the sheep.

PC3 distinguished between behavioural measurements at weaning and those later at post-weaning and pre-slaughter. This indicates that timing of measurement impacts the score received and the repeatability of the measure. This can be seen in the correlation matrix; subjective crate score and CV of weight had low correlations between weaning and post-weaning but were moderately correlated across time between post-weaning and pre-slaughter (Table 6.2). Similarly, correlations of objective and subjective flight speed measurements were also better between post-weaning and pre-slaughter, as compared to between weaning and post-weaning, indicating greater repeatability in older lambs. This may be due to differences in the length of the intervals between behavioural measurements (longer interval between weaning and post-weaning) or a result of the increased age and experience of the lambs. When the interval between behavioural observations is short, it is more likely that the animals are in a similar physiological state and experiencing similar environments. In contrast, over longer intervals, particularly when the animals are young, there is more opportunity for developmental change such as sexual maturation, changes in environment such as seasonal shifts, and habituation to the procedure (Bell *et al.*, 2009). Similar results have previously been reported regarding the repeatability of flight speed in cattle, where higher correlations between measures of flight speed were found the closer in time the measures were (Petherick *et al.*, 2009a). Age may have an additional effect in this case; in humans behavioural consistency improves with age, probably as a result of accumulated experience with the environment (Roberts and DelVecchio, 2000). This is supported by evidence in sheep of greater repeatability of behaviour in older animals and poor repeatability for animals that were naïve to the testing protocol (Horton *et al.*, 2009). Thus PC3 likely represents increasing experience of sheep, resulting in reduced reactivity.

In comparison to wether lambs, ewe lambs were less reactive overall (PC1) and displayed greater divergence in reactivity between restrained tests and flight speeds (PC2) and between weaning and later measures (PC3), though only the latter effect was significant at $P<0.05$. Female lambs had slightly higher agitation and slightly lower flight speeds than males, resulting in a higher score for PC2. This sex effect on agitation has been seen previously (Dodd *et al.*, 2013) and it is possible that this greater reactivity during isolation from conspecifics indicates a greater social motivation in females. In cattle, heifers that were more socially active displayed greater movement and vocalisation, both traits which were measured in the agitation test, when isolated from pen-mates (Boissy and Bouissou, 1995). Furthermore, sex differences have also been found in the cortisol response to isolation, with females displaying a greater response than males (Hernandez *et al.*, 2010).

Females were more reactive at weaning than males and less reactive at later measurements. Greater reactivity in females compared to males has been demonstrated repeatedly in sheep (Vandenheede and Bouissou, 1993; Viérin and Bouissou, 2003; Boissy *et al.*, 2005; Hernandez *et al.*, 2010). However, it would appear in the present study that females habituate faster to the test than males, resulting in a re-ranking of the sexes at later testing periods. Cognitive behavioural testing in lambs has suggested that female lambs are faster learners than male lambs prior to 18 months of age, likely due to differences in rates of maturation (Hernandez *et al.*, 2009a). Thus it is possible that female lambs learn more quickly not to fear isolation and handling by humans.

A significant effect of breed on PC1 was seen, with 2nd cross lambs displaying lower scores and slower flight speeds than their 1st cross counterparts. Breed differences in behaviour have been found in several studies (Le Neindre *et al.*, 1993; Boissy *et al.*, 2005; Starbuck *et al.*, 2006). These breed effects may reflect differences in the way the stimulus is perceived by lambs of varying genetic lines, resulting in different levels of fear and reactivity. There are also differences between breeds in their reaction to stressful stimuli, with some breeds displaying an active coping mechanism (high levels of locomotion, high-pitched bleats, escape attempts) and others a passive mechanism (immobilisation, quiet bleating, retreat from stimuli) (Boissy *et al.*, 2005).

There was a negative relationship between average daily gain and PC2, indicating that slower growing lambs had greater differences between flight speeds and restrained test scores, though this was not statistically significant (P=0.07). There was no effect of weaning weight on any of the components of behaviour, nor was there any effect of handling treatment. One of the objectives of this research was to assess whether behavioural studies conducted in the Cooperative Research Centre for Sheep Industry Innovation's "Information Nucleus" flocks could be generalised to the commercial population of sheep given the high frequency of handling that occurs in these research flocks. The lack of significant handling effect suggests that the behaviour of "research flock" lambs in previous studies (Lennon *et al.*, 2009; Hocking Edwards *et al.*, 2011; Plush *et al.*, 2011; Dodd *et al.*, 2013; 2014) is representative of similar sheep in a commercial setting; the increase in frequency of handling was insufficient to create behavioural differences in these sheep.

Heart rate was only assessed during the weaning testing period. Although it was possible to find the heart rate of older lambs, the process was time consuming as the heart sounds of the lambs because more difficult to distinguish in the larger animals. There was no relationship between heart rate at weaning and the principal components describing behaviour. This is supported by previous results in goats where significant differences in heart rate could not be established between goats whose vocal behaviour indicated differing levels of fear (Lyons and Price, 1987). However, correlations of heart rate and heart rate variability have been found with behaviour in sheep when continuous heart rate monitoring was used (Wickham *et al.*, 2012), suggesting that although heart rate at a given moment was not informative, continuous measures may give different results.

There were no relationships between final salivary cortisol (adjusted for initial cortisol) and the components describing behaviour, though initial cortisol at post-weaning was significantly associated with PC1 and PC2. This relationship indicates that lambs with a more reactive temperament, and particularly lambs with higher restrained scores, had lower salivary cortisol prior to behavioural testing at post-weaning. In studies that induced long term stress, increases (McNatty and Young, 1973), decreases (Fordham *et al.*, 1991) and no change (Hawken *et al.*, 2013) in basal cortisol level have all been reported. If lambs which are more reactive in the present study are experiencing chronic stress due to regular handling, this may explain the observed differences in initial salivary cortisol. More evidence is needed to understand whether inherently reactive sheep are predisposed to chronic stress from normal management activities.

The lack of relationships between adjusted final salivary cortisol, representing cortisol response, and the components describing behaviour may be a result of the large variability of hypothalamic-pituitary-adrenal axis activity. Cortisol secretion can be influenced by a wide variety of factors, including the length of time to the last meal, weather and physiological state (Mormède *et al.*, 2007). Variation in time to collect the final saliva sample would also have induced a potentially large degree of variability in saliva cortisol. In addition, responses to environmental cues and stressors vary between individuals. Thus in this field-based experiment designed to mimic commercial sheep growing conditions any relationships which exist between the behaviours tested and cortisol response were obscured, although in an experiment utilising genetically homogenous lambs in a highly controlled environment such relationships may be more easily discovered should they exist. More frequent sampling may also be necessary to detect differences in cortisol secretion between more and less reactive lambs (Rushen, 1991).

Lambing paddock accounted for a small amount of variance in PC3, and may indicate early life effects on the lambs which impact on behavioural reactivity at weaning but not at later stages. Maternal environment, including postnatal ewe behaviour and prenatal stress and nutrition, have been demonstrated to affect lamb behaviour (Hawkins *et al.*, 1999; Hawkins *et al.*, 2000a; Hawkins *et al.*, 2000b; Hawkins *et al.*, 2001; Erhard *et al.*, 2004; Roussel-Huchette *et al.*, 2008). These effects may have influenced to a small extent the behaviour of the lambs in this study, although it would appear that these were not significantly large to impact overall reactivity.

6.5 Conclusion

Temperament can be illustrated using a combination of restrained and non-restrained tests. These different types of tests measure different aspects of temperament, likely representing differences in the perception of the stressful stimulus by the sheep. The timing of measurement affects behavioural reactivity, with measures at weaning differing from those conducted later in life, likely due to sheep experience. Further work is necessary to determine the relationships between reactivity and stress in lambs, and there is also scope to continue these measures in older sheep.

Chapter 7. Facial hair patterning is not a useful indicator trait for behavioural reactivity in lambs

During review of the literature, it became apparent that hair patterning could be used as an indicator trait for behaviour in other species, notably cattle. Since behaviour can be time consuming to measure and is better measured some months after weaning rather than in younger lambs, an indicator trait could make identification of sheep that are likely to be more reactive during handling easier at an early age, allowing the implementation of management strategies to minimise handling stress on these sheep. This chapter has been prepared as a manuscript for submission to *Applied Animal Behaviour Science*.

Lambs gathered for assessment of facial hair patterning during behavioural testing at Struan Research Centre, South Australia, December 2011.

Authorship Statement

Cathy Burnard was responsible for data collection and analysis, writing the manuscript and was corresponding author.

23rd June 2014

Signature Date

Wayne Pitchford supervised the development of this work and assisted with statistical analysis and in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Susan Hazel supervised the development of this work and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Janelle Hocking Edwards supervised the development of this work and data collection and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Abstract

Facial hair patterning is related to behaviour in a number of species including cattle, horses, dogs and humans. Face cover score, a subjective measure of facial hair patterning in sheep, is highly heritable and repeatable, and so is a suitable candidate for assessment as an indicator trait. Additionally, it is possible to describe facial hair patterning in sheep using the position of the line at which the wool on the head stops and is replaced by smooth hair over the face (the hairline). This study examined the usefulness of face cover score and hairline position as indicators for a variety of measures of behavioural reactivity in 251 lambs born to Merino and Border Leicester x Merino dams by Poll Dorset sires. Behavioural and facial hair patterning measures were conducted seven days after weaning (85 to 145 days of age), at post-weaning (221 to 255 days of age) and within three weeks of slaughter (259 to 320 days of age). Behaviours assessed include agitation (movement of the lamb while visually isolated), objective and subjective measures of movement in the weigh crate, and objective and subjective measures of exit speed from the weigh crate (flight speed). Face cover was moderately repeatable (0.30), as were hairline position on both ratio (0.42) and ordinal scales (0.39). There were no significant relationships between measures of facial hair patterning and behaviour, demonstrating that these measures are not suitable indicator traits for behavioural reactivity in lambs.

7.1 Introduction

Temperament is defined as an animal's inherent potential to respond in a particular way to a stressful stimulus (Dodd *et al.*, 2012). Inherent temperament is determined by genetic and permanent environmental effects such as early life experience and does not change over time, but its expression is influenced by the animal's temporary environment (Bates *et al.*, 1995) and physiological status (Viérin and Bouissou, 2001). The expression of temperament in an animal is its behavioural reactivity, and this is the trait which is commonly the aim to measure. However, measuring behaviour can be difficult and time consuming. An indicator trait which is easy to assess, highly heritable and correlated with the behaviour of interest would have value in the selection of animals to improve temperament.

Facial hair patterning, specifically rostral-caudal and lateral hair whorl position, has been demonstrated to be related to behaviour in a number of species including cattle, horses, dogs and humans (Grandin *et al.*, 1995; Randle, 1998; Lanier *et al.*, 2001; Gorecka *et al.*, 2007; Olmos and Turner, 2008). Similarly, links have been found between facial hair pattern and lateralisation in multiple species (Tanner *et al.*, 1994; Randle and Elworthy, 2006; Murphy and Arkins, 2008; Tomkins *et al.*, 2012) including humans (Klar, 2003; Weber *et al.*, 2006; Beaton and Mellor, 2007). It has been hypothesised that the relationship between hair patterning and behaviour is due to the concurrent processes of hair formation and brain development *in utero*. The angle of hair follicles is a result of the direction of stretch and tension on the skin exerted by the maturation of the foetal brain and thus hair patterning may be useful as an indicator of the size or shape of the brain (Smith and Gong, 1973; Smith and Gong, 1974; Furdon and Clark, 2003).

Face cover, a measure of facial hair patterning in sheep, is highly heritable, with estimates ranging from 0.29 to 0.45 (Mortimer and Atkins, 1993; Lewer *et al.*, 1995; Mortimer *et al.*, 2009). The score is also highly accurate, with a between-scorer repeatability of 0.85 (Fail and Dun, 1956) and repeatability over time of 0.36-0.49 (Young *et al.*, 1960). As sheep do not commonly exhibit a facial hair whorl, face cover may be an alternative descriptor of facial hair patterning for investigation as an indicator trait for behaviour. Additionally, the line at which the wool on the head stops and is replaced by smooth hair over the face (the hairline) may also be a valid descriptor. This study examined the usefulness of face cover score and hairline position as indicators for a variety of measures of behavioural reactivity in lambs.

7.2 Methods

7.2.1 Animals

This trial utilised 251 lambs, born between 15 July and 13 September 2011 to Merino and Border Leicester x Merino dams by Poll Dorset sires (1st and 2nd cross respectively). These lambs were progeny of sheep bred within the Cooperative Research Centre for Sheep Industry Innovation's "Information Nucleus" flock at Struan Research Centre, South Australia (van der Werf *et al.*, 2010). Lambing occurred across six paddocks and lambs were collected into a single flock at the end of lambing. Lambs were weaned at 78 to 138 days of age, 11 weeks after the end of lambing. Lambs were grown on green dryland annual pastures until weaning and then irrigated pastures (annual ryegrass/clover) until slaughter. This project was approved by the Animal Ethics Committees of the University of Adelaide and Primary Industries and Resources of South Australia.

7.2.2 Measurements

Behavioural and facial hair patterning measures were conducted seven days after weaning (85 to 145 days of age), at post-weaning (221 to 255 days of age) and within three weeks of slaughter (259 to 320 days of age). Weaning and pre-slaughter behavioural measures were collected over two consecutive days and post-weaning measures were collected over three consecutive days. During all testing external noises were minimized and no dogs were permitted near the sheep to reduce the impact of these stressors on the behaviour of the sheep.

Agitation of the lamb was measured using an isolation test. The lamb was restrained within a fully enclosed box with dimensions 1.5m x 0.7m x 1.5m (Blache and Ferguson, 2005). The number of vibrations caused by movement and vocalisation of the lamb over 30 seconds were measured using an "agitation meter". The agitation meter was calibrated using a standard "electro-sheep" (Blache and Ferguson, 2005). After the agitation test, the lamb was released into a section of raceway for holding. Agitation testing was conducted on groups of approximately 20 lambs prior to beginning the next test on the first lamb. While in the raceway, face cover was scored. Face cover is a subjective score which refers to the amount of wool cover on the face, including the top of the head and the jowls (Fail and Dun, 1956; Anonymous, 2007). Scores range from 1: open face with no wool in front of the ears and top knot or on the jowls, to 5: heavy wool growth over the entire face with wool from the top and side of the muzzle joining. Video footage was taken of each lamb's face for later assessment of hairline position. From this footage between two and seven still images were captured. Still images were chosen to give a standard angle between the camera and the lamb's skull; the head was down so that the line between the nostrils was not visible but the pink of the nose above this was, ears were forward perpendicular to the head and the camera was positioned centrally over the head (Figure 7.1). The position of the hair line was determined on both ratio and ordinal scales (Olmos and Turner, 2008). For the ratio scale, a horizontal line was drawn across the forehead between the points at which the ventral aspects of the ears joined the head, and another across the nose between the apexes of the nostrils. The distance between these lines was assigned a value of 100 arbitrary units, with zero being positioned on the forehead and 100 on the nose. Using this scale the hairline position was determined as a percentage of the distance below the line of the ears (Figure 7.1). On the ordinal scale, hair line position was recorded as being above, in line with or below the level of the eyes (Olmos and Turner, 2008).

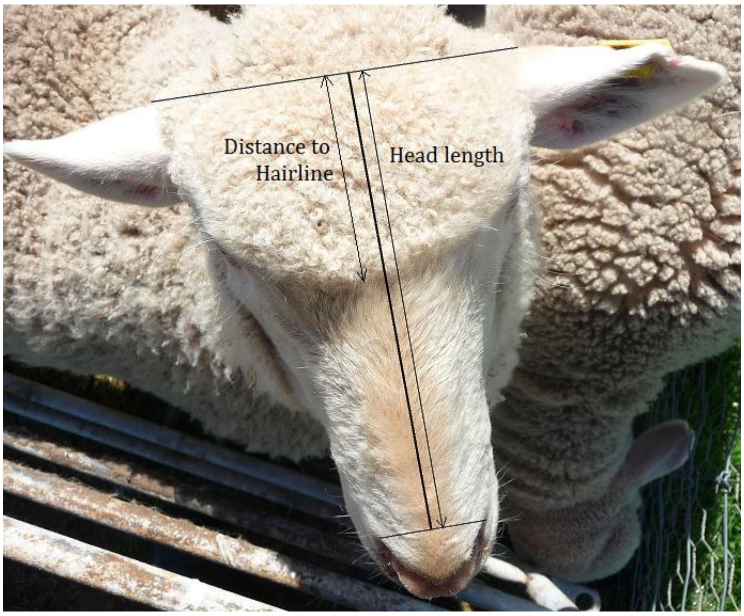


Figure 7.1. Determination of distance to hairline and head length for calculation of hair line position on the ratio scale.

Each lamb then entered the weigh crate where weight and the coefficient of variation (CV) of weight were recorded using Agitation v2.01 software (Horton and Miller, 2011) in conjunction with a Tru-Test XR3000 weigh unit (Tru-Test Ltd., New Zealand). The CV of weight was calculated by the software using 10 weights per second, over 20 seconds for a total of 200 weights per lamb. During this time (20 sec) a subjective crate score was also assigned to the lamb, ranging from 1: Calm, no movement, to 5: Constant movements and shaking the crate (Pajor *et al.*, 2008).

The lamb was then released from the weigh crate and allowed to leave without interference or prompting. Flight speed was the average speed at which a lamb travels a 1.7m distance on exit from the weigh crate. Flight time was measured using infra-red start and stop beams set 1.7m apart and attached to a timer. This time was then divided by distance to yield flight speed (Burrow and Dillon, 1997). A subjective score of walk, trot, run or leap was also allocated to the exit behaviour of each lamb, based on the method used in cattle (Hoppe *et al.*, 2010).

7.2.3 Statistical analyses

Although facial hair patterning measures were conducted at three time points, only the post-weaning and pre-slaughter measures were analysed as per Meat and Livestock Australia recommendations that lambs are measured at a minimum of four months of age (Anonymous, 2007). Very young lambs are known to have woollier faces, and face cover scores at younger ages are less repeatable than those measured later (Archer *et al.*, 1982). Analyses of facial hair patterning measures were conducted in ASReml version 3 (Gilmour et al., 2009) using the univariate model below:

$$FP_{ijklmnop} = \mu + S_i + B_j + W_k + G_l + T_m + T_m x S_i + T_m x B_j + T_m x W_k + T_m x G_l + L_n + P_o + e_{ijklmnop}$$

where $FP_{ijklmnop}$ is the given facial hair patterning measure (face cover, ordinal hair line, ratio hair line), for lamb (L) n, from lambing paddock (P) o (6 paddocks), of sex (S) i (ewe, wether), breed (B) j (1st or 2nd cross), with weaning weight (W) k, average daily gain (G) l at testing time (T) m (post-weaning or pre-slaughter), with a residual or error of $e_{ijklmnop}$, where S, B, W, G, T and the interactions of T with S, B, W and G are fixed effects and L and P are random. This model was used to test significance for each of the fixed effects, in addition to the proportion of variance attributable to lambing paddock (P/(L + P + e)), and repeatability (L/(L + P + e) of flight speed (Falconer, 1981). The effects of growth rate and time were included to test whether growth of the lamb and wool changed the measurement or scoring of these traits. A significance threshold of $P<0.05$ was used for the fixed effects.

As described in Chapter 6, the five behavioural measures were subjected to a principal components analysis. Three components explained 54% of the variance in these behavioural measures and these were retained for analysis with facial hair patterning measures. Component one (PC1) had positive loadings for all behaviours and was interpreted as temperament. Component two (PC2) had positive loadings for restrained tests (agitation, subjective crate score and CV of weight) and negative loadings for objective and subjective flight speed, indicating context-specific behavioural reactivity. Component three (PC3) had primarily positive loadings for measures at weaning (0.08-0.54) and primarily negative loadings for post-weaning and pre-slaughter measures (-0.57-0.19), and indicated the difference in reactivity resulting from time of measurement. The relationships between facial hair patterning measures and principal components describing behaviour were assessed with regressions against each component:

$$PC_{ijklmno} = \mu + S_i + B_j + W_k + G_l + F_m + P_n + e_{ijklmno}$$

where sex (S), breed (B), weaning weight (W) and average daily gain (G) were fixed effects fitted prior to the average across post-weaning and pre-slaughter for each facial hair patterning trait (F). A random effect of lambing paddock (P) was also included.

7.3 Results

All three facial hair patterning measures were moderately repeatable (Table 7.1). Lambing paddock accounted for 7-11% of total variance in facial hair patterning. There was a significant interaction of breed and time of measurement on face cover (P=0.01), with 2nd cross lambs having a significant increase in face cover score from post-weaning to pre-slaughter while 1st cross lambs did not (Figure 7.2). Similarly, the interaction of breed and time was significant for ratio hair line position (P=0.05). At post-weaning, 1st and 2nd cross lambs were significantly different from each other, with 2nd cross lambs exhibiting hair lines higher on the face, i.e. having less face cover and a lower ratio hair line position. At pre-slaughter this difference had disappeared (P>0.05).

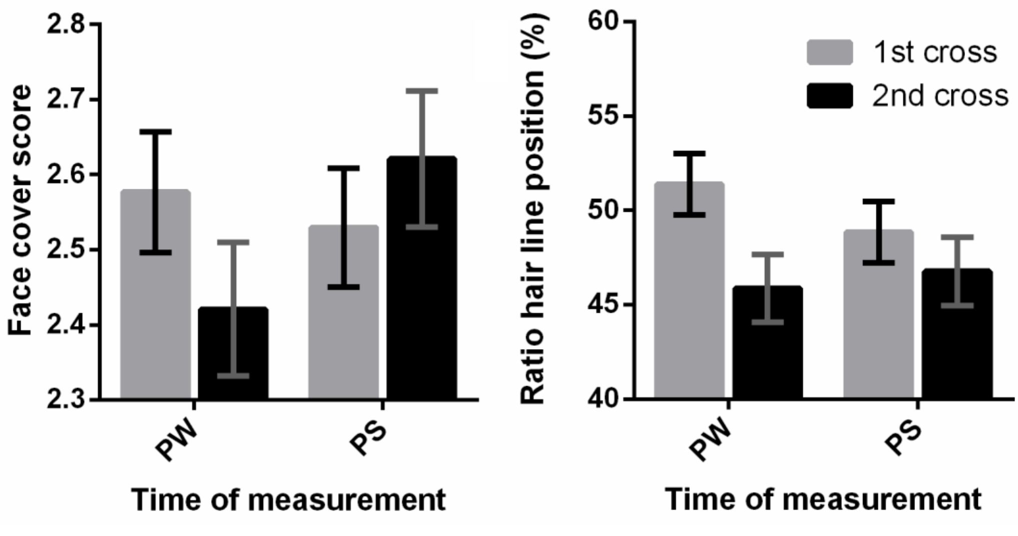


Figure 7.2. Breed effect on face cover and ratio hair line position in lambs at post-weaning (PW) and pre-slaughter (PS).

The interaction of average daily liveweight gain and time was significant for both ratio and ordinal hair line positions (P=0.01). At post-weaning, there was no relationship between hair line position on both scales and average daily gain.

However, at pre-slaughter higher rates of average daily gain were associated with hair lines higher on the face.

Face cover score, ratio hair line position and ordinal hair line position were not significantly related to any of the three principal components describing behaviour (Table 7.1).

Table 7.1. Tests of significance (probabilities) of face cover and hair line positions on principal components describing behaviour, and repeatability of facial hair patterning measures.

	Face cover	Ratio hair line	Ordinal hair line
PC1: Temperament	0.86	0.35	0.87
PC2: Behavioural reactivity	0.18	0.94	0.06
PC3: Sheep experience	0.78	0.26	0.68
Repeatability across time	0.30	0.42	0.39

7.4 Discussion

This study examined the usefulness of face cover score and hairline position as indicators for a variety of measures of behavioural reactivity in lambs. Face cover, ratio hair line position and ordinal hair line position were moderately repeatable. In combination with high estimates of the heritability of face cover reported in other studies (Mortimer and Atkins, 1993; Mortimer *et al.*, 2009; Dodd *et al.*, 2013), this indicates that these traits may be good candidates for genetic selection. However, no significant relationships were found with the principal components describing behaviour, demonstrating that these measures of facial hair patterning are not suitable indicator traits for behavioural reactivity in lambs. The relationship between context-specific behavioural reactivity (PC2) and ordinal hair line was almost significant (P=0.06). Lambs with hairlines below the eyes tended to have higher values for PC2, indicating that they had greater differences between flight speeds and restrained test scores. This is not sufficient evidence to suggest that facial hair patterning should be pursued as an indicator for behaviour in sheep.

Comparatively, other species have shown relationships between hair patterning and behaviour. In cattle, the rostral-caudal location of the facial hair whorl is correlated with behaviour in the crush, yards and auction ring. Cattle with whorls located above the eyes have more variable behaviour and are more agitated during handling (Grandin *et al.*, 1995; Lanier *et al.*, 2001; Olmos and Turner, 2008). The response of cattle to an unfamiliar human, in terms of flight distance and levels of interest shown, is also associated with hair whorl location (Randle, 1998). A similar result is seen in horses, with a high whorl predicting a lesser degree of manageability compared to individuals with whorls in line with or below the eyes (Gorecka *et al.*, 2007). The lateral whorl position has also been related to behaviour in equines. Ponies with facial whorls located to the left of the midline were described as calmer, friendly and more enthusiastic, whereas those with whorls to the right were more wary and flighty (Randle *et al.*, 2003).

Hair whorl position in species such as cattle and horses describes the pattern of hair, likely caused by the pressure exerted on developing follicles by brain growth in utero (Furdon and Clark, 2003). In comparison, face cover and hair line position are describing regional differentiation in pelage. Long 'wool' fibres, as on the body of the sheep, are the result of follicles which remain in the anagen (growth) phase for long periods of time, whereas the shorter fibres found on the face, limbs or belly of the sheep have a shorter anagen phase followed by a longer telogen (quiescent) phase (Scobie *et al.*, 2006). These differences in the hair follicle cycle are likely to have a different prenatal origin to hair whorl position and direction, and thus do not exhibit the same relationships with behaviour as that found in other species.

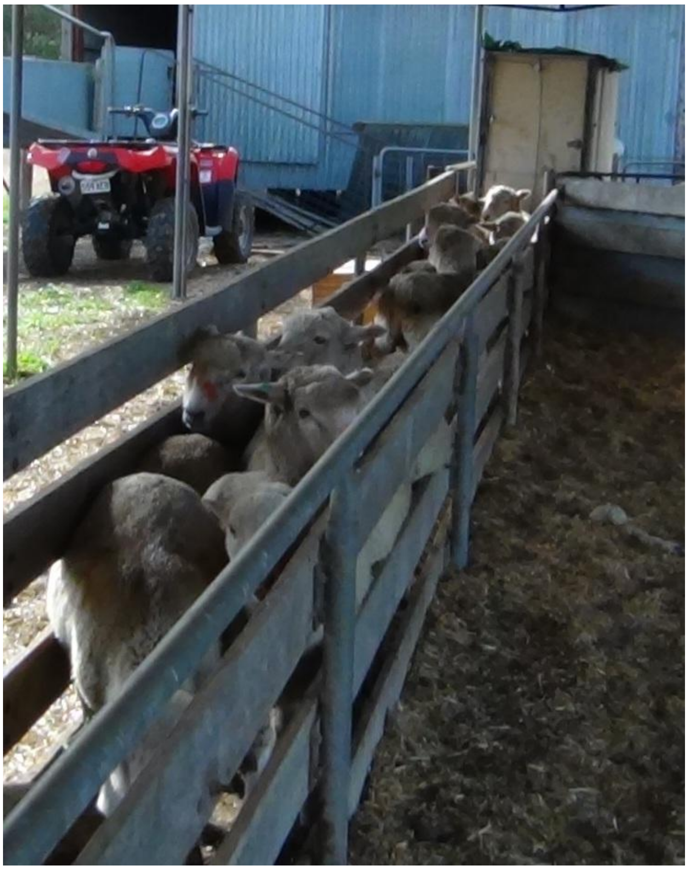
There were significant breed by time effects on both face cover score and ratio hair line position. It is likely that the lower sensitivity of the ordinal hair line position is the reason for the breed effect not being significant for this trait. In both face cover score and ratio hair line position, 1st cross lambs had a drop in the measurement and 2nd cross lambs had a rise, though the scale of these changes is dependent on the measure. For face cover score the increase in score in 2nd cross lambs was significant and this likely represents wool growth over the period. On the ratio scale, hair line position was significantly different between breed types, with 2nd cross lambs displaying a hair line higher on the face (lower value of the measure). This was expected as the 2nd cross lambs were 25% Border Leicester, a breed with an entirely open (no wool) face, with a lower proportion of the woollier faced Merino breed. These results indicate that wool growth over a relatively short period (36 days) can have significant effects on the assessment of face cover which should be accounted for when evaluating animals.

At pre-slaughter, higher rates of average daily gain were associated with hair lines higher on the face on both ratio and ordinal scales. This relationship between an "open face" and greater growth has been demonstrated repeatedly (Drinan and Dun, 1967; Cockrem, 1968; Bottomley, 1982; Matebesi *et al.*, 2009; Mortimer *et al.*, 2009). It has been suggested that this relationship may be due to the effect of wool blindness on the ability of the lamb to graze (Fail and Dun, 1956), although that is unlikely to be the cause in this trial. There is potential for this to be a genetic effect; lambs with lower genetic potential for growth also inherit a woollier face (Drinan and Dun, 1967). In this case, Border Leicesters are known to have higher growth rates than other breeds and a more open face, and there is also likely to be a heterosis effect increasing growth rates of the 2nd cross lambs (Fogarty and Mulholland, 2012).

In conclusion, although facial hair patterning exhibits characteristics of a good indicator trait, such as ease of classification and moderate repeatability, there were no significant relationships between measures of facial hair patterning and behaviour. This demonstrates that these measures are not suitable indicator traits for behavioural reactivity in lambs.

Chapter 8. Behavioural reactivity is predictive of stress at slaughter and subsequent meat quality in lambs

Chapter 8 investigates the relationships between the principal components describing behavioural reactivity formed in Chapter 6, and carcass and meat quality. This chapter has been prepared as a manuscript for submission to *Livestock Science*.



Sheep at Struan Research Centre during behavioural testing, April 2012.

Authorship Statement

Cathy Burnard was responsible for data collection and analysis, writing the manuscript and was corresponding author.

23rd June 2014

Signature Date

Wayne Pitchford supervised the development of this work and assisted with statistical analysis and in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Susan Hazel supervised the development of this work and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

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Janelle Hocking Edwards supervised the development of this work and data collection and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Abstract

Stress in lambs prior to slaughter affects post-mortem muscle metabolism, primarily through changes to the rate of pH decline. This can result in decreased meat quality. It is thus important that pre-slaughter stress levels are managed to ensure appropriate post-mortem muscle metabolism. Understanding the relationship between behavioural reactivity, an animal's behavioural response to stress, in lambs on the farm and their subsequent physiological response to the slaughter process may assist in the prediction of meat quality. This study investigated the relationships between measures of behavioural reactivity and carcass and meat quality attributes in lamb.

Behavioural measures were conducted on 251 lambs seven days after weaning (average age 104 days), at post-weaning (average age 240 days) and within three weeks of slaughter (average age 280 days). Behavioural measures included agitation while visually isolated, a subjective crate score, coefficient of variation of weight and objective and subjective flight speeds. Lambs were then slaughtered and carcass characteristics including weight, loin muscle dimensions, fatness, pH decline and meat colour collected.

Lambs that were more reactive during behavioural testing, particularly with reference to flight speed, demonstrated elevated pre-slaughter cortisol, glucose and lactate, and subsequently lower loin pH 24 hours post-mortem. Although after relatively short periods off feed, these lambs displayed lower loin pH 24 hours post-mortem, lambs that are more reactive during behavioural testing may be at higher risk of elevated ultimate loin pH if the time off feed is greater. These results indicate that

characterisation of sheep based on reactivity in behavioural tests may have positive effects for prediction of subsequent meat quality.

8.1 Introduction

Meat quality is a complex parameter and can be affected by post-mortem muscle metabolism, specifically pH decline, which is in turn influenced by pre-slaughter muscle glycogen levels (Pethick and Rowe, 1996). Chronic stress prior to slaughter depletes muscle glycogen levels, resulting in a slow pH decline and meat which enters rigour at lower than optimal temperatures. This combination of slow glycolytic rate and low muscle temperature causes the muscle to cold-shorten and become tough (Savell *et al.*, 2005). High ultimate pH due to reduced glycogen levels also results in meat which is dark in colour, dry in the mouth and more susceptible to microbial growth (Pethick and Rowe, 1996; Ferguson and Warner, 2008). It is thus important that pre-slaughter stress levels are managed to ensure appropriate post-mortem muscle metabolism. Understanding the relationship between behavioural reactivity, an animal's behavioural response to stress, in lambs on the farm and their subsequent physiological response to the slaughter process may facilitate the characterisation of sheep based on their behavioural reaction to handling and assisting in the prediction of meat quality.

A number of tests of behavioural reactivity have been utilised in sheep. These include measures of movement and vocalisation during social isolation (Blache and Ferguson, 2005), subjective and objective measures of movement in the weigh crate

(Pajor *et al.*, 2008; Horton and Miller, 2011) and exit (flight) speed from the weigh crate (Blache and Ferguson, 2005; Hoppe *et al.*, 2010). In beef cattle, faster flight speeds and increased movement in the weigh crate have been associated with significant reductions in growth rates, carcass weight and meat tenderness, colour and pH (Burrow and Dillon, 1997; Voisinet et al., 1997a,b; King et al., 2006; Petherick et al., 2009b).

However, studies of the relationships between behavioural reactivity and carcass traits in sheep are limited, using few behavioural measures and only shortly after weaning (Dodd *et al.*, 2014). The aim of this paper was to investigate the relationships between measures of behavioural reactivity and carcass and meat quality attributes in lamb. Plasma cortisol, glucose and lactate have been used to indicate stress levels of lambs at slaughter.

8.2 Methods

8.2.1 Animals

This trial utilised 251 lambs, born between 15 July and 13 September 2011 to Merino and Border Leicester x Merino dams by Poll Dorset sires (1st and 2nd cross respectively). These lambs were progeny of sheep bred within the Cooperative Research Centre for Sheep Industry Innovation's "Information Nucleus" flock at Struan Research Centre, South Australia (Fogarty *et al.*, 2007; van der Werf *et al.*, 2010). Lambing occurred across six paddocks and lambs were collected into a single flock at the end of lambing. The maximum difference in ages of lambs was 60 days. Lambs were weaned at 78 to 138 days of age, 11 weeks after the end of lambing. For the purposes of another trial, lambs were randomly assigned to High- or Low-Handling treatment groups seven days after weaning. Groups were balanced for weaning weight, sex and dam breed. After weaning, Low-Handling lambs were yarded only for necessary management procedures, such as shearing and animal health procedures including drenching, vaccination and crutching, and for behavioural testing. High-Handling lambs were also yarded for these procedures but were additionally handled through the yards approximately every 3 weeks in order to test the effect of frequent handling on behavioural reactivity. Lambs were grown on green dryland annual pastures until weaning and then irrigated pastures (annual ryegrass/clover) until slaughter. A subset of lambs (145), selected on weight and balanced across handling groups, was slaughtered at 280 to 340 days of age. On the day prior to slaughter, lambs were transported to the abattoir lairage. Carcasses were electrically stimulated and trimmed according to AUS-MEAT specifications (AUS-MEAT, 2005). This project was approved by the Animal Ethics Committees of the University of Adelaide and Primary Industries and Resources of South Australia.

8.2.2 Measurements

Behavioural measures were conducted on lambs seven days after weaning (average age 104 days), at post-weaning (average age 240 days) and within three weeks of slaughter (average age 280 days). Weaning and pre-slaughter behavioural measures were collected over two consecutive days and post-weaning measures were collected over three consecutive days. During testing external noises were minimized and no dogs were permitted near the sheep to reduce the impact of these stressors on the behaviour of the sheep.

Agitation of the lamb was measured by placing it in a fully enclosed isolation box with dimensions 1.5m x 0.7m x 1.5m (Blache and Ferguson, 2005). The number of vibrations caused by movement and vocalisation of the lamb over 30 seconds were measured using an agitation meter. The agitation meter was calibrated using a standard "electro-sheep" (Blache and Ferguson, 2005). After the agitation test, the lamb was released into a section of raceway for holding. Agitation testing was conducted on approximately 20 lambs prior to beginning the next test on the first lamb.

Weight and coefficient of variation (CV) of weight were recorded for each lamb using Agitation v2.01 software (Horton and Miller, 2011) in conjunction with a Tru-Test XR3000 weigh unit (Tru-Test Ltd., New Zealand). The CV of weight was calculated by the software using 10 weights per second, over 20 seconds for a total of 200 weights per lamb. During this time (20 sec) a subjective crate score was assigned to the lamb, ranging from 1: Calm, no movement, to 5: Constant movements and shaking the crate (Pajor *et al.*, 2008). The lamb was then released from the weigh crate and allowed to leave without interference or prompting. Time to pass between two infra-red start and stop beams (1.7m apart) attached to a timer was recorded and flight speed was calculated (m/s) (Burrow and Dillon, 1997). A subjective score of walk, trot, run or leap was also allocated to the exit behaviour of each lamb, based on the method used in cattle (Hoppe *et al.*, 2010).

A subset of lambs (145) was slaughtered at 280 to 340 days of age. On the day prior to slaughter, lambs were transported to abattoir lairage. Lambs were off feed for a total of approximately 40 hours prior to slaughter. Exsanguination blood samples were collected from 47 of the lambs into potassium oxalate sodium fluoride tubes for glucose and lactate concentration determination, and into lithium heparin tubes for cortisol determination. Hot carcass weight (HSCW) was recorded prior to chilling.

pH decline, eye muscle dimensions, fat depth and meat colour were measured on the first 98 carcasses to reach the chiller. Declines were calculated from pH readings at approximate carcass temperatures of 35 °C, 18 °C and 12 °C and at approximately 24 hours post mortem (pH_{24}), from the left portion of the *M. Longissimus thoracis et lumborum* (LL) muscle at the caudal end over the lumbar–sacral junction. The first reading was taken as soon as the carcasses reached the chiller using a TPS WP-80 pH meter and a glass-body, spear-tipped probe (Ionode IJ44C). Muscle temperature was measured using a stainless steel cylindrical probe attached to the pH meter. The pH meter was calibrated before use and at regular intervals, using pH 4.0 and pH 6.8 buffers at ambient temperature for all measurements. A linear regression was used to describe the relationship between temperature and pH for each carcass and estimate pH at 18°C (pH18) and temperature at pH=6 (pH6temp). Estimated pH6temp was used to assess whether the pH decline of each carcass fell within the pH-temperature window, achieving pH=6 at between 18°C and 35°C (Pearce *et al.*, 2010; van de Ven *et al.*, 2013). A further pH measurement was taken 24 hours post mortem in the *M. semitendinosus* (pH_{24}ST). Carcasses were cut between the 12th and 13th ribs approximately 20h after slaughter. Fat depth and eye muscle width (EMW) and depth (EMD) were measured using callipers. After exposure to air for 30 minutes to allow samples to "bloom", fresh meat colour was measured using a Minolta Chromameter Model CR-300 recording lightness (cfL*), redness (cfa*) and yellowness (cfb*).

8.2.3 Metabolite analyses

Cortisol levels in plasma samples were analysed using an ELISA test (DetectX Cortisol EIA kit, Arbor Assays, USA) validated for sheep plasma. Samples were run in triplicate. The cortisol ELISA has a sensitivity of 17.3pg/mL, and all samples tested had intra-assay coefficients of variation of less than 10% and a between-sheep coefficient of variation of 55%. The quantitative determination of plasma glucose and lactate was performed with a Roche Cobas Integra 400 plus clinical chemistry analyser using the Glucose HK Gen. 3, and the Lactate Gen. 2 assay kits, with the Calibrator f.a.s., and quality controls: PreciControl ClinChem Multi 1 and PreciControl ClinChem Multi 2 (Roche Diagnostics GmbH, Mannheim, Germany). The coefficient of variation was less than 2.5% for each assay with each QC.

8.2.4 Statistical analysis

Analyses were conducted using ASReml version 3 (Gilmour *et al.*, 2009) and R (R Core Team, 2013; Revelle, 2013). Behaviours were subjected to a principal components analysis as described in Chapter 6. Three components explained 54% of the variance in these behavioural measures and these were retained for analysis with carcass measures. Component one (PC1) had positive loadings for all behaviours and was interpreted as temperament. Component two (PC2) had positive loadings for restrained tests (agitation, subjective crate score and CV of weight) and negative loadings for objective and subjective flight speed, indicating context-specific behavioural reactivity. Component three (PC3) had primarily positive loadings for measures at weaning (0.08-0.54) and primarily negative loadings for post-weaning and pre-slaughter measures (-0.57-0.19), and indicated the difference in reactivity resulting from time of measurement, likely due to sheep experience.

The relationships between carcass measures, metabolites (plasma cortisol, glucose and lactate) and principal components describing behaviour were assessed with regressions against each carcass measure or metabolite:

$$C_{ijklmn} = \mu + S_i + B_j + W_k + PC_l + P_m + e_{ijklmn}$$

where $C_{ijklmno}$ is the given carcass measure or metabolite, for a lamb from lambing paddock (P) m (6 paddocks), of sex (S) i (ewe, wether), breed (B) j (1st or 2nd cross), with carcass weight (W) k, with a residual or error of e_{ijklmn}, where S, B and W are fixed effects and P is random. PC_l represents the principal component describing behaviour for which the relationship to behaviour was being assessed, and was fitted as a fixed effect. These same regressions were also fitted replacing the PCs with three behaviours which were strongly correlated with the PCs to assist with interpretation: subjective flight speed (post-weaning) for PC1, agitation (post-weaning) for PC2 and subjective flight speed (weaning) for PC3.

Although all significant results are reported, due to the large number of regressions being performed and the subsequent increased risk of Type I errors, only results where $P<0.01$ were considered significant.

8.3 Results

Three of the 98 carcasses measured did not achieve $pH_{24}LL$ of less than 6 (Table 8.1). Of the 98 carcasses on which pH was measured, pH decline could be calculated on only 50. Nineteen of these (38%) fell outside the pH-temperature window, with 7 carcasses recording a pH6 temperature of less than 18°C and 12 carcasses recording temperatures above 35°C. A total of 29 carcasses had pH decline and plasma cortisol records; 23 had pH decline and plasma cortisol, lactate and glucose.

Table 8.1. Summary statistics of carcass composition, lamb quality and physiological indicators of stress in 1st and 2nd cross Merino lambs.

	Count	Mean	SD	Min	Max
Carcass composition					
HSCW (kg)	145	23.2	4.1	17.1	35.9
EMW (mm)	98	60.2	3.5	51.8	69.3
EMD (mm)	98	30.1	3.0	23.5	38.1
Fat (mm)	98	2.54	1.30	0.52	6.65
Objective eating quality					
pH6temp (°C)	50	27.7	9.4	2.1	38.5
pH18	95	5.71	0.26	5.31	6.58
$pH_{24}LL$	98	5.67	0.15	5.4	6.12
$pH_{24}ST$	98	5.98	0.21	5.63	6.62
cfL*	98	35.3	3.0	28.0	42.8
cfa*	98	21.2	2.4	15.6	27.2
cfb*	98	4.1	1.6	0.5	8.0
Physiological indicators of stress					
Glucose (mM/L)	36	3.91	0.49	3.07	4.90
Lactate (mM/L)	36	2.07	0.95	0.91	4.64
Cortisol (nM/L)	47	55.5	30.4	10.9	140.7

HSCW = hot carcass weight; EMW = eye muscle width; EMD = eye muscle depth; pH6temp = temperature at pH=6; pH18 = pH at 18°C; $pH_{24}LL$ = pH of *Longissimus thoracis et lumborum* approx. 24 hours post mortem; pH24ST = pH of *M. semitendinosus* approx. 24 hours post mortem; cfL* = meat lightness; cfa* = meat redness; cfb* = meat yellowness.

There was a significant relationship between PC1 and plasma cortisol, explaining 18% of the variance in cortisol, with lambs with a more reactive temperament (high reactivity in restrained tests and faster flight speeds) having higher cortisol at slaughter (Table 8.2). Plasma cortisol was also related to $pH_{24}LL$ such that lambs with higher cortisol had lower loin pH 24 hours post-mortem. There was also a trend seen between PC1 and $pH_{24}LL$ whereby more reactive lambs had lower loin pH 24 hours post-mortem, although this was not significant.

Increasing plasma glucose and lactate at slaughter were associated with lower loin pH 24 hours post-mortem (Table 8.2). There was a trend towards higher lactate in lambs with lower PC2 scores (faster flight speeds, less reactive in restrained tests) explaining 5% of the variance in lactate, and lower PC2 scores were also associated with lower $pH_{24}LL$, although these effects were not significant.

There was a significant relationship between PC3 and loin width (EMW) such that lambs that were less reactive at weaning, more reactive at post-weaning and pre-slaughter, or both, had larger loins. There were no significant effects of the principal components describing behaviour or of the metabolites indicating stress on the remaining carcass composition traits, pH decline or meat colour.

There were no significant relationships between the individual behaviours chosen to represent the PCs and the carcass traits or stress metabolites. There were no effects of sex or carcass weight on the stress metabolites. Breed affected blood cortisol levels, with 1st cross lambs having much higher cortisol at slaughter (63.9nM/L ± 6.4) than 2nd cross (39.4nM/L ± 8.6, P=0.029).

Table 8.2. Tests of significance (F-probabilities) of principal components describing behaviour and physiological indicators of stress on carcass traits and physiological indicators.

	PC1	PC2	PC3	Glucose	Lactate	Cortisol
HSCW	0.76	0.09†	0.46	1.00	0.98	0.96
EMW	0.42	0.32	0.01**	0.42	0.41	0.75
EMD	0.22	0.61	0.76	0.69	0.98	0.85
Fat	0.48	0.49	0.79	0.67	0.74	0.78
pH6temp	0.22	0.99	0.15	0.73	0.48	0.18
pH18	0.99	0.47	0.89	0.62	0.43	0.38
pH_{24}LL	0.08†	0.10†	0.55	<0.001***	<0.001***	0.01**
pH_{24}ST	0.26	0.86	0.97	0.51	0.86	0.31
cfL*	0.68	0.76	0.83	0.20	0.23	0.90
cfa*	0.64	0.43	0.76	0.24	0.16	0.13
cfb*	0.76	0.26	0.92	0.10†	0.10†	0.20
Glucose	0.84	0.93	0.63		0.02*	0.98
Lactate	0.21	0.04*	0.15	0.03*		0.37
Cortisol	0.004**	0.62	0.12	0.91	0.57	

†P<0.10, * P<0.05, ** P<0.01, *** P<0.001. HSCW = hot carcass weight; EMW = eye muscle width; EMD = eye muscle depth; pH6temp = temperature at pH=6; pH18 = pH at 18°C; pH_{24}LL = pH of *Longissimus thoracis et lumborum* approx. 24 hours post mortem; pH24ST = pH of *M. Semitendinosus* approx. 24 hours post mortem; cfL* = meat lightness; cfa* = meat redness; cfb* = meat yellowness.

8.4 Discussion

The aim of this paper was to investigate the relationships between behavioural reactivity and carcass and meat quality attributes in lambs. Physiological measures have been used to indicate stress levels of lambs during behavioural testing and at slaughter. Lambs that were more reactive during behavioural testing had higher plasma cortisol at slaughter and lower loin pH 24 hours post-mortem. This has been demonstrated previously (Deiss *et al.*, 2009). The pH of muscle in living animals is around 7.2, but after slaughter this falls as lactic acid accumulates due to anaerobic glycolysis (Gardner *et al.*, 2001). The extent of this fall is dependent on muscular glycogen stores; if muscle glycogen is low prior to slaughter, pH will remain high (Gardner *et al.*, 2001). Stress triggers the mobilisation of muscle and liver glycogen reserves (Visser *et al.*, 2008), resulting in the accumulation of lactate in the muscle pre-slaughter and a reduced initial pH. In lambs with adequate glycogen stores, stress immediately prior to slaughter may result in accelerated pH decline as lactic acid production would begin prior to slaughter but with insufficient time to be cleared from the muscle before the cessation of circulation (D'Souza *et al.*, 1998). Thus, by the time the carcass is measured at 24 hours post-mortem, lactic acid production has been occurring for longer than in the unstressed lamb.

Lambs with a more reactive temperament (PC1) experienced increased stress in lairage resulting in elevated plasma cortisol and lower loin pH 24 hours post mortem. This is consistent with the finding that lambs displaying lower loin pH 24 hours post-mortem also exhibited elevated plasma glucose and lactate at exsanguination. In combination, these results suggest that lambs that are more reactive during behavioural testing, particularly with reference to flight speed, are likely to experience greater stress at the abattoir resulting in pre-slaughter metabolism of glycogen and subsequently lower loin pH 24 hours post-mortem providing glycogen stores are sufficient. This is likely to have been an acute stress immediately prior to slaughter during movement to the stunning area. Lambs in this study were off feed for approximately 40 hours. Under low stress conditions time off feed does not result in glycogen depletion (Escribano *et al.*, 2014). However, if lambs are experiencing physical or psychological stress, glycogen stores in the muscles will decline over time with no opportunity for replenishment (Jacob *et al.*, 2009). Although after relatively short periods off feed more reactive lambs displayed lower loin pH 24 hours post-mortem, these lambs may be at higher risk of elevated ultimate loin pH if the time off feed is greater, or nutrition and thus muscle glycogen stores are lower.

There was a significant relationship between PC3 (age specific reactivity) and loin width (EMW) with lambs that were less reactive at weaning, more reactive at post-weaning and pre-slaughter, or both, had larger loins. This was not a breed effect, as breed was accounted for within the statistical model. A negative association between reactivity and growth has been demonstrated previously in sheep, where lambs that were calmer as measured by a crate score and objective flight speed were heavier at the end of fattening than nervous lambs (Pajor *et al.*, 2008). Similarly in cattle, less reactive animals achieved greater growth rates and carcass weights (Burrow and Dillon, 1997; Müller and von Keyserlingk, 2006). This may be due to increased feed intake or reduced energy cost for stress or avoidance behaviours of animals that are generally more settled.

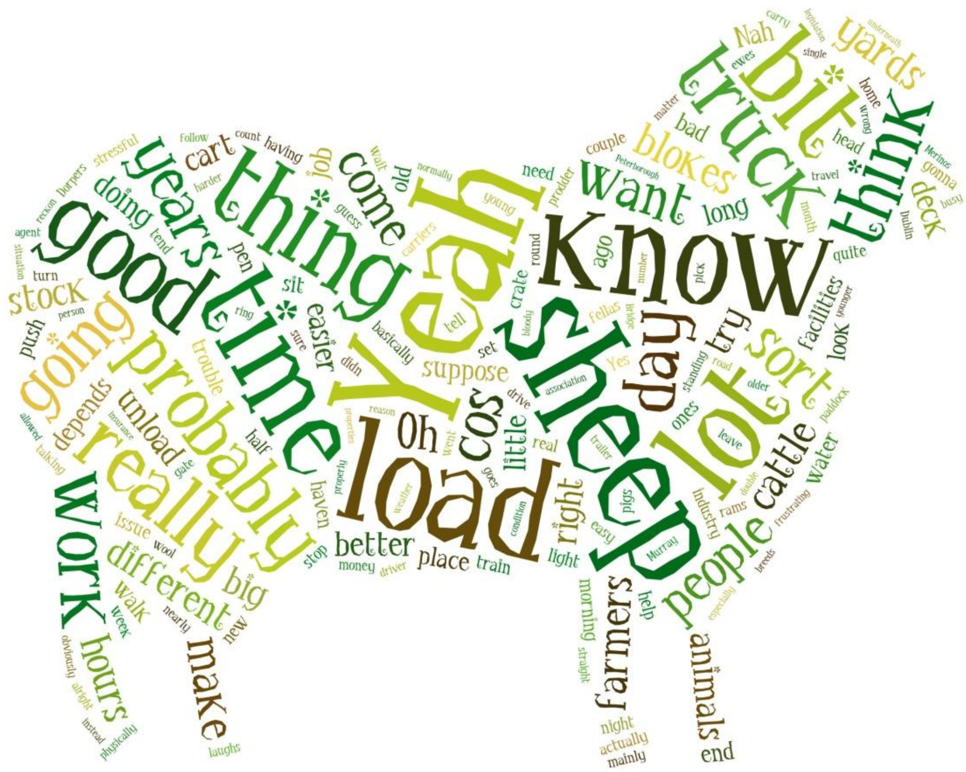
Breed significantly impacted plasma cortisol, with 1st cross lambs having much higher cortisol at slaughter than 2nd cross lambs. These 1st cross lambs also had greater overall reactivity (PC1, Chapter 6.3); this result serves to strengthen the evidence that higher overall reactivity is indicative of greater stress.

8.5 Conclusion

The aim of this paper was to investigate the relationships between behavioural reactivity and carcass and meat quality attributes in lamb. Lambs that were more reactive during behavioural testing, particularly with reference to flight speed, are likely to experience greater stress at the abattoir resulting in elevated pre-slaughter cortisol, glucose and lactate, increased metabolism of glycogen and subsequently lower loin pH 24 hours post-mortem. Although after relatively short periods off feed these lambs displayed lower loin pH 24 hours post-mortem, lambs that are more reactive during behavioural testing may be at higher risk of elevated ultimate loin pH if the time off feed is greater. These results indicate that characterisation of sheep based on reactivity in behavioural tests may have positive effects for prediction of subsequent meat quality. Understanding the relationships between behavioural reactivity and carcass quality will allow producers to selectively manage lambs to improve both welfare and carcass quality, by targeting highly reactive lambs with nutritional and handling treatments to ensure minimal stress and maximal glycogen reserves in those lambs most likely to be stressed by the slaughter procedure.

Chapter 9. Facilities, breed and experience affect ease of sheep handling: the livestock transporter’s perspective

Chapter 9 explores a social component of sheep behaviour; the perceived importance of factors affecting ease of handling, and the interactions between these from the perspective of the stockman. This chapter has been prepared as a manuscript for submission to *Animal*.



Word cloud created from the interview transcripts used in this study. Word size indicates frequency of use (larger = more frequent).

Authorship Statement

Cathy Burnard was responsible for data collection and analysis, writing the manuscript and was corresponding author.

23rd June 2014

Signature Date

Wayne Pitchford supervised the development of this work and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Susan Hazel supervised the development of this work and assisted in qualitative analysis and editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Janelle Hocking Edwards supervised the development of this work and assisted in editing the manuscript. Consent is given for Cathy to present this article for examination towards the Doctor of Philosophy.

23rd June 2014

Signature Date

Abstract

Animals which are difficult to handle require more labour and are more prone to injury, affecting both profitability and welfare of the livestock. This highlights the importance of understanding the factors that impact on ease of handling. As the handling of livestock involves people, there is a social component. It is also understood that many other factors may contribute to animal behaviour during handling, although traditionally these factors have been assessed in isolation under experimental conditions. The aim of this study was to gain a deeper understanding of the perceived importance of factors affecting ease of handling, and the interactions between these from the perspective of the stockman.

Qualitative interviews were used to investigate the factors affecting sheep behaviour during handling. Interviews were directed to livestock transporters as this group observe and experience the phenomenon of sheep handling on a regular basis, with a range of sheep varying in sex, condition, age, breed, previous experience and physiological status, and a similarly diverse set of conditions, including weather, location and design of yards, and availability of helpers. Interview transcripts underwent thematic analysis.

Livestock transporters discussed the effects of attitudes and behaviours towards sheep, helpers, facilities, distractions, environment, dogs and a variety of sheep factors including breed, preparation, experience and sex on sheep behaviour during handling. Transporters repeatedly demonstrated care and professionalism in stating that patience and experience were key factors determining how a person might deal with difficult sheep, and described a need for formal training available to

the industry. Livestock transporters strongly believed facilities had the greatest impact, followed by sheep experience and breed. Transporters also discussed the effects of distractions, time of day, weather, dogs, other people, sheep preparation, body condition and sex on ease of handling. The concept of individual sheep temperament was indirectly expressed.

9.1 Introduction

Animals which are difficult to handle require more labour and are more prone to injury (Gregory, 2008), affecting both profitability and welfare of the livestock. This highlights the importance of understanding the factors that impact on ease of handling. In a quantitative survey of French Limousin cattle breeders, farmers believed the most important factors in ease of handling were regular contact with the handler and good facilities followed by genetic factors (Boivin *et al*., 2007). However, no such study, either quantitative or qualitative, has been conducted previously investigating the factors that affect ease of handling in sheep.

As the handling of livestock involves people, there is a social component, and it has been documented in sheep (Coleman *et al.*, 2012), cattle (Breuer *et al.*, 2000; Hemsworth *et al.*, 2000; Hemsworth *et al.*, 2002; Mounier *et al.*, 2008; Coleman *et al.*, 2012) and pigs (Hemsworth *et al.*, 1989) that attitudes held by stockmen contribute to the behaviour of the stock. It is also understood that many other factors may contribute to animal behaviour during handling, although traditionally these factors have been assessed in isolation under experimental conditions (Romeyer and

Bouissou, 1992; Viérin and Bouissou, 2003; Boissy *et al.*, 2005). The use of traditional quantitative approaches to understand this social phenomenon was deemed unlikely to be adequate as there have not been previous studies in this area, so a qualitative research approach was used. Qualitative research interviews aim to explore and describe a spectrum of attitudes and experiences within a field, rather than presenting a representative sample attempting to quantify opinions (Patton, 1990). Through a qualitative approach, researchers are able to capture the social aspects of a phenomenon in order to improve understanding of how people perceive the relationships between variables (Patton, 1990). The aim of this study was to gain a deeper understanding of the perceived importance of factors affecting ease of handling, and the interactions between these from the perspective of the stockman.

9.2 Methods

9.2.1 Selection of participants

Interviews were directed to livestock transporters as this group observe and experience the phenomenon of sheep handling on a regular basis. Sheep farmers are likely to only handle their own flock, representing a limited number of breeds, classes (e.g. pregnant/non-pregnant, whole male/wether/ewe, sick/healthy, shorn/full wool) and individuals in a limited set of circumstances (e.g. yard design, weather conditions, time of day). In contrast, livestock transporters are faced with an array of animals, varying in sex, condition, age, breed, previous experience and physiological status, and a similarly diverse set of conditions, including weather, location and design of yards, and availability of helpers. Thus, interviews were conducted with livestock transporters to utilise their wide and varied experience to gain a deeper understanding of sheep handling. It is acknowledged that transporters generally only handle sheep during loading and unloading of trucks and so the deep understanding gained from these interviews is of a relatively narrow range of handling operations.

The Livestock Transporters Association of South Australia was asked to identify livestock transporters who would be willing to be interviewed. The six transporters represented four geographical regions of South Australia: the far north and west coast (n=2), mid north (n=2), upper south east (n=1) and lower south east (n=1). All primary interviewees were male. Family members of the primary interviewee were present for two of the six interviews, and participated in one. While six seems a small number, new interviews were conducted until the point of saturation, when no new information or opinions were arising from the data (Glaser and Strauss, 1967; Patton, 1990). This occurred relatively quickly so it was deemed that six interviews were able to provide representative and rich information.

9.2.2 Qualitative semi-structured interviews

The interviews followed a semi-structured interview guide and lasted an average of one hour (range 18-95 minutes). Immediately before the interview, participants were given both verbal and written summaries of the study and were asked to sign a consent form as approved by the University of Adelaide Human Research Ethics Committee. Interviews used the general opening question ‘Could you tell me about your business?' and progressed through questions about the transporters experiences of handling sheep during loading and unloading of trucks. Open ended questions were used to investigate the perceived relative importance of factors including facilities, breed, previous handling, weather and farmer attitudes in the ease of handling of sheep. Triangulation was achieved by asking a variety of non-specific questions to allow livestock transporters to raise issues without prompting (Table 9.1).

9.2.3 Transcript analysis

All interviews were recorded, transcribed in full and coded using NVivo 10 (Anonymous, 2012b). The process of analysis was iterative and cyclical; ideas developed during transcription and coding of early interviews informed subsequent interviews. A thematic analysis of the transcripts was conducted using a grounded theory approach, whereby codes were identified as they arose from the data, rather than using a pre-defined list (Glaser and Strauss, 1967). An open coding technique was used, examining each transcript in detail to identify as many codes as possible. These were then categorised under a series of "parent codes" when a main theme was identified. Cross-checking of coding by a second researcher was used to achieve analytical triangulation (Patton, 1990).

9.3 Results

From the interviews, six transcripts totalling 58,933 words were coded under nineteen codes, forming four main themes describing factors that affect ease of handling of sheep (Figure 9.1). Five additional codes grouped answers to questions that were not issue-specific, used by the interviewer to allow livestock transporters to raise issues without prompting (Table 9.1) and to achieve triangulation of sources by checking for consistency of what transporters said over time within the interview (Patton, 1990). The following section describes the ideas and opinions of the livestock transporters captured within the themes identified, raised both when prompted and without prompting (Figure 9.2).

The transporters interviewed had all been carting livestock for at least ten years, with two transporters having carted for more than thirty years. Four of the six transporters interviewed got their stock handling experience from a family farm prior to taking up work as a livestock transporter; the remaining two learned on the job. Regardless of where their experience was obtained, the transporters believed that good stock handling skills develop over time from experience and that this learning was ongoing.

Figure 9.1. The hierarchy of themes generated using a grounded theory approach to coding of livestock transporter interviews on factors affecting the ease of handling of sheep.

Table 9.1. Non-specific questions used to create unprompted discussion of factors affecting ease of sheep handling.

Code	Main question
Factors affecting handling	What affects how easy it is to load sheep?
Desired change	If there was one thing you could change to make sheep handling easier, what would it be?
Unloading easier or harder	Is unloading easier or harder than loading and why?
Other comments	Do you have any other comments on factors that affect sheep handling?
Biggest stress	What is the most stressful part of handling sheep?

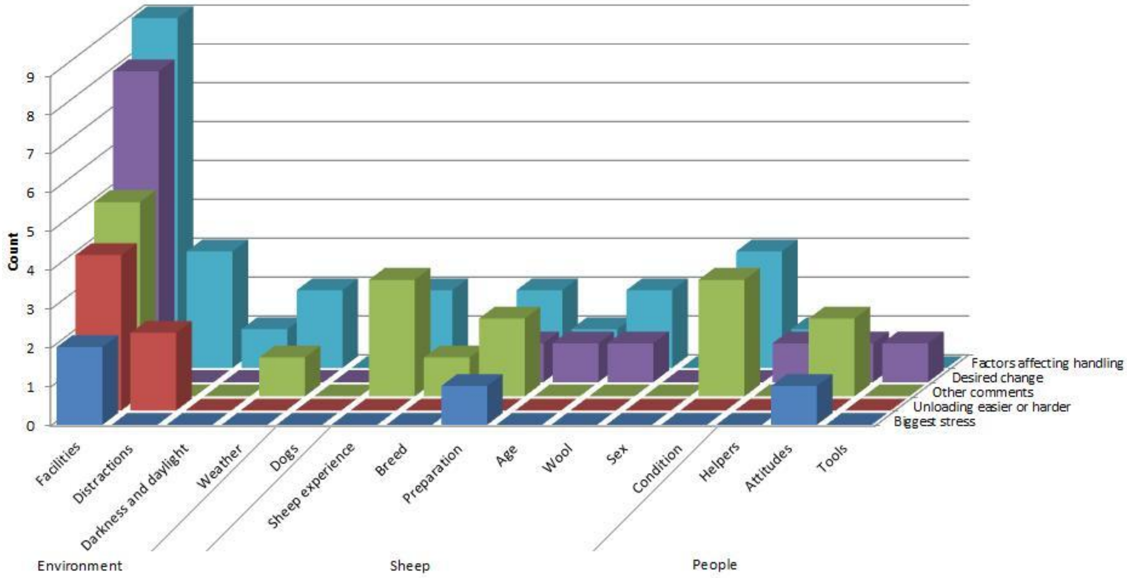


Figure 9.2. The frequency with which livestock transporters, <u>without prompting</u>, mentioned each effect on the ease of handling of sheep when asked a variety of non-specific questions (see Table 1 for explanation of questions).

9.3.1 People

9.3.1.1 Attitudes towards sheep

<u>Positivity</u>

The livestock transporters interviewed all had positive attitudes towards sheep, stating that they loved and derived enjoyment from their jobs.

"I just love doing it so that's why I keep doing it."

Transporters stated that they preferred carting livestock over other jobs due to its interesting and variable nature. They also appeared to hold livestock transporting in esteem:

"But it's an honourable job, I like it and I'd encourage anyone to do it."

<u>Anthropomorphism</u>

Anthropomorphism was common to all of the interviews, with up to 5% of each interview transcript being coded to that topic. Transporters spoke of the sheep being reluctant, stubborn and having wants, and similarities were drawn between the motivations of sheep and humans.

> *"Animals feel it just the same as we feel it, you don't feel like doing much at 2 o'clock in the afternoon on a 40 degree day."*

Human values, such as laziness and friendship, were commonly used to explain the behaviours of sheep.

> *"Once you've got them loaded they're pretty comfortable, sitting in a group they feel safe in their own environment like with their own friends, they feel quite safe"*

> *"up our way they don't have to walk very far and they're too fat and lazy I think and they just don't want to load."*

Although the transporters generally did not appear aware of the concept they were framing, and most at some stage personified the sheep, attributing them with a human-like consciousness.

> *"You have different situations and different personalities in sheep and stuff like that, same as humans I suppose."*

<u>Patience and experience</u>

When asked to define ‘difficult sheep’, transporters described animals that refused to move or were particularly stubborn, but did not identify these as a source of stress to themselves. When asked how they respond to difficult sheep, transporters believed that persistence was the only option:

> *“Well you’ve just got to persevere with them, that’s about it, because it doesn’t make any difference what you do, they won’t go. Sometimes you’ll try a different situation, like if you’ve got a ramp there and they don’t want to run up there, sometimes you change where they come into the ramp and if it’s around a corner, they get around a corner and they think oh we’re on the ramp already, it’s too late, they’re on there. So you just gotta think like a sheep really, that’s what they say. Just think, if they don’t want to go this way, try a different way and it might work. But sometimes it doesn’t matter which way you try, you just gotta push one up the ramp and that’s it.”*

Overall there was a strong indication from transporters that patience and experience were key factors in determining how well a person might deal with difficult sheep and that a lack of these attributes resulted in sheep being more difficult to handle (five of six transporters).

9.3.1.2 Behaviours towards sheep

Temper

Although transporters acknowledged that sheep handling can sometimes be frustrating, it was a consistently held view (three of six transporters) that behaviours associated with losing their temper, such as yelling and swearing, generally had negative results, making the stock harder to handle and wasting time and energy:

> *"An old farmer, this is an old fella, said to me one day, he said 'Look, you may feel a lot better after you do that, I would too' he said 'but to be honest with you, that's only one, you've got another 680 standing out there looking at you, waiting for you to put them on that truck'. So he said, 'No point in doing your Charlie son, you might as well just plod along, if it takes you an hour longer, so be it' he said, 'but at the end of the day you're not going to be as worn out'. And he was right."*

A negative view of those who resorted to violence was common. Transporters indicated that people with short tempers or who physically abused the sheep were not retained in the transport industry, and that inexperienced livestock transporters were the most likely to perform these kinds of unacceptable behaviours. The livestock transporters demonstrated their professionalism and care for sheep by condemning poor attitudes and violent behaviour towards sheep.

Prodders

The use of tools such as electric prods and sheep rattles was not mentioned by transporters without prompting. When asked about prodder use, four of six transporters indicated that electric prodders are widely used in the sheep transport industry. One transporter initially denied that they used prodders at all but went onto describe some situations in which they did. Some transporters were uncomfortable discussing prodder use and felt that they needed to defend the practice. Transporters suggested that although prodders are used on most loads of sheep, they are used on very few sheep per load, and are most often used when turned off. All of the transporters agreed that used correctly, prodders were a valuable tool.

> *"to me a simple shock on the backside of a sheep will scoot it up to the front of the truck but you can sit there for ten minutes with someone with a stick or a piece of poly pipe trying to move the sheep along, it's a lot worse for the sheep and for us again. Used the right way they're a good tool."*

Transporters also described the problems associated with misuse of prodders in regards to the behaviour of the sheep. They indicated that overuse led to stubborn sheep and emphasised the importance of prodding the sheep only in the correct locations on the body and when the sheep is facing in the direction it needs to run.

Other tools

Other tools were rarely mentioned. Four of the six transporters indicated that sheep rattles are a common tool for transporters but sticks and poly pipes were not used by transporters.

> *"They're farmer tools [sticks and poly pipes]. Carriers don't use them. There's a lot of rattlers and shakers and that sort of thing around. […] Mostly the carriers, the people that deal with it all the time on a daily basis have the right tools."*

9.3.1.3 Helpers

Transporters indicated that the people assisting during handling, which included farmers, farm hands and stock agents, were usually helpful, having a positive impact on ease of sheep handling (five of six transporters). However, two transporters noted that if the helper had a negative attitude, described as being "*set in their ways*" or "*just expect it to happen then and there and don't really want to do the work*", this could make sheep handling harder, though they were not explicit in the precise impact this had on sheep behaviour.

9.3.2 Environment

9.3.2.1 Facilities

Transporters were unanimous in declaring facilities, particularly loading ramps, as the biggest single factor influencing the ease with which they handled sheep (Figure 9.2).

> *"I'd say the facilities would be 90% of the ease and the whole dynamic of the job. If you've got good yards, good facilities, good ramp, the job can be pleasurable. But if you haven't it can be a nightmare."*

Transporters often brought up this issue in response to the question 'what affects how easy it is to load sheep?'

> *"... having good loading facilities for a start. That's probably the biggest thing, loading ramps, forcing yards, yeah."*

It was also reiterated by three of the six transporters when asked whether they found unloading easier or harder than loading:

> *"Depends on the facilities. They can run on really good, pen up really good and then depending on where you've gotta unload they've got to walk down a steep ramp or there might be mesh that they can see through and there's light shining under it and they just won't run, yeah it can depend. You hope that they run off better, but yeah it just depends on the facilities."*

Four of the six transporters also suggested improvement of facilities when asked 'if there were anything you could change to make sheep handling easier, what would it be?'

> *"The ramps are the biggest factor, for sure. Yeah, if I could have a good ramp at every place I loaded and unloaded my work load would be cut in half, for sure."*

Old, poorly designed and poorly maintained yards and ramps were described as not only causing immediate difficulty in handling sheep, but also resulting in sheep that were harder to handle in the long term, after they learn that they can escape from inadequate yards. Size and shape of pens, fence height, position of gates, and flooring material were some of the issues raised with regards to yard design. The most commonly cited problem with ramps was that they were too short and too steep, servicing only the lower deck of the truck and forcing transporters to use the steep internal truck ramps (five of six transporters). Transporters indicated that use of ramps that were too steep caused sheep to be more reluctant to move up and down ramps. Additionally, participants described ramps that were in a state of serious disrepair, with rotted floors and held together with fencing wire or twine.

9.3.2.2 Distractions

Distractions, such as shadows and noises were described as another very important factor influencing sheep handling. In particular, the reflection of light on surfaces such as pools of water and aluminium ramps and decks was seen as a strong influencing factor on how well sheep would run (five of six transporters).

"you might get sun glare off of a ramp or something that they'll baulk at"

Other distractions described included position of shadows, novel surfaces such as bitumen, steel and mesh, and noises such as dogs barking.

9.3.2.3 Darkness and daylight

Time of day was perceived to be moderately important by most (four) of the transporters. There was a strong preference for working in daylight, and the observation was made by these transporters that sheep are more difficult to move in the dark.

"Obviously daylight is a huge, natural light, natural daylight is so superior to artificial light because of the shadows and the casts and sheep don't like running in the dark."

Two of the six transporters indicated that if it was necessary to work stock after dark, having lights was critical but that positioning of these lights is also important to avoid poorly placed shadows. In contrast, one transporter observed that some breeds of sheep, particularly Dorpers, move better at night if there are no lights, although he allowed that working in daylight was still the preference. When working during the day, four of the six transporters indicated that the angle of the sun relative to the facilities was of importance to avoid distracting reflections or shadows. In hot weather, early morning or late afternoon were the preferred times for working livestock to avoid exhaustion in the sheep which also impacted on ease of handling.

9.3.2.4 Weather

Hot weather was perceived to be less of a problem for sheep handling than cold or wet weather (three of six transporters). Transporters observed that wet sheep are hesitant to move and reluctant to touch each other, making them more difficult to handle in inclement weather. Only two of the six transporters mentioned wind; both of these dismissed the effect as of minor importance.

9.3.3 Dogs

Livestock transporters did not mention dogs without prompting and were generally dismissive of their impact (Figure 9.2). The consensus was that a good dog can make handling sheep much easier but the presence or absence of a dog does not appreciably impact on the behaviour of the sheep.

> *"If you've got a good dog it'll certainly make life easier. But it's not the end of the world if you haven't got one."*

However, the effectiveness of dogs interacted with a number of other factors, including breed, sheep experience and sex. Three of the six transporters indicated that Dorpers and Damaras were highly averse to dogs and the presence of dogs made these breeds much more difficult to handle. Previous experience with dogs was important, with sheep that were naive to dogs being described as "*more spooky*" when worked with a dog (two of six transporters). Two transporters also noted that rams tend to attack dogs and that it was therefore harder to work rams with a dog.

9.3.4 Sheep

9.3.4.1 Sheep experience

After facilities, sheep experience and breed were the most important factors influencing ease of sheep handling. Transporters described a balance between not enough and too much experience in sheep. They preferred sheep that had been handled or trucked at least once or twice before to those that had never been handled such as very young lambs, as naive sheep appeared not to know what was expected of them (five of six transporters). Similarly, older sheep that had experienced minimal handling, such as the self-shedding breeds that do not require shearing, were also described as difficult to handle for the same reasons. Five of the six transporters also observed that over-handled sheep, especially hand-raised lambs, were very difficult to handle as they were too used to people.

> *"If they're over-handled they tend to be a bit sooky, they won't […] do what you want. Under-handled, well same thing. They sort of find that happy medium, they'll run, they'll get the gist of what they've got to do. If they've been trucked a couple of times they generally run up alright, don't have to push them."*

9.3.4.2 Breed

Although breed was only raised once without prompting (Figure 2), transporters emphatically indicated a strong breed component to the behaviour of sheep (six of six transporters), differentiating Merinos, White Suffolks and cross-bred sheep from Dorpers and Damaras.

"The Dorpers and Damaras, a lot of the South African bred sheep really huddle together and are very hard to move on and off of trucks. The traditional cross-breds and Merinos that we've got here, we don't have too much problems."

In general, transporters found Merinos and cross-breds easier to handle (four of six transporters). In contrast, Dorpers and Damaras were stated as being more difficult to handle, and one transporter postulated that this was at least partly due to the much lower amount of handling received by these self-shedding breeds as a result of not being shorn and generally being raised on large extensive operations. However, transporters also observed that the behaviour of the different breeds during handling was varied (four of six transporters). Damaras were described as a strongly flocking breed that tended to move as tight groups, as compared to Merinos which moved in a much looser flock. Transporters (three of six) generally felt that Dorpers and Damaras were much easier to work without dogs, as dogs tended to make these breeds more stubborn and reluctant to move, whereas dogs generally made movement of Merinos and cross-breds much easier.

9.3.4.3 Preparation, age and condition

A number of closely interacting sheep factors were reported as minor impacts on the ease of handling, including the preparation and curfew of the sheep (six of six transporters), age (six of six transporters) and body condition (five of six transporters). Transporters suggested that sheep that were not curfewed from feed and water for a sufficient period prior to loading tended to be slower and more stubborn during handling and more often lay down in the truck during transport.

> *"Yeah they can be a bit harder to load. And you can tell, you can hear them rattling when they're walking up the ramp, belly sloshing around. They get a bit slower I suppose, and probably a little bit more stubborn at times."*

Some transporters attributed this to the extra weight of feed and water in the gut making the sheep "*weak in the legs*". Half of the transporters identified weakness generally as a problem for handling, indicating that weak sheep were more difficult to move, more inclined to sit down during handling and transport and had more trouble walking up and down steep ramps. Overly fat sheep, very underweight sheep and older sheep were recognized as those most likely to be weak.

9.3.4.4 Wooliness

A high degree of wooliness was reported to be a problem for handling by four of the six transporters, in part due to the effects of wool blindness on the sheep's willingness to move. Sheep in full wool were reported to be reluctant to move close to another sheep, and one transporter described the wool as "*Velcro*" for its tendency to make moving sheep past each other difficult.

9.3.4.5 Sex

Transporters never raised the topic of sheep sex without prompting (Figure 9.2) and when asked about the impact of sex on handling, three of the six transporters stated that it was not important. One transporter indicated that rams could be more aggressive, and two observed that rams would attack working dogs rather than moving away from them as ewes and wethers did. Wethers were perceived to be behaviourally the same as ewes. Overall transporters believed that sex was not a significant factor in the ease of sheep handling.

9.3.4.6 Unpredictability

In addition to the factors described above, the transporters described some innate characteristic of sheep that gave a degree of uncertainty to their handling. Each transporter expressed this slightly differently, but the overall concept was that even if all the circumstances in which sheep are handled, such as facilities, breed, and weather, are known, it is impossible to predict with certainty how each individual will behave. This was a theme that pervaded the interviews, with each opinion on the factors described above given with the caveat that there are no guarantees or hard-and-fast rules.

"Yeah you can have a good run or you can have a bloody bad run, so. Nothing's really ... you can never really predict it."

Overall the transporters described the importance of the individual sheep in the process of handling, using statements such as *"You get your good and your bad"* and *"It all depends on the animal itself"*, and this appeared to be independent of the other factors described.

9.3.5 Training

A lack of formal training available to the industry, either by employers or as part of a formal course, was described by the majority of interviewees. Some participants expressed a desire to see an accredited stock handling course developed for the livestock transport industry and for this to be part of a broader quality assurance program.

> *"It would be good if someone could train some of the younger fellas really early in their career. I know it's pretty hard to do cos it's a one on one thing, but getting them young fellas… It's such a hard job if you're not prepared for or you don't know what you're doing, yeah. And there's really no training whatsoever, for people entering it, you know. It's really just a matter of going with the boss, and a lot of lads now say here's your truck, you know, it's got 18 gears and the sheep are up the road, I'll see you later. And it's really tough for the lads that don't know what they're doing. I've seen fellas that I've felt sorry for, you know what I mean. They get to the point where they're almost in tears and going to chuck it away. But it doesn't need to be like that."*

There was a degree of scepticism regarding the content of such a course as some transporters felt that the complexity of sheep behaviour would mean that not enough material could be covered in any kind of formal course. Interviewees agreed that any training had to be practical and one suggested that training for new transporters could involve spending a set amount of time in a truck with a more experienced transporter. Another participant recommended that the choice of trainer would also be important, indicating that someone perceived to be young and inexperienced would be poorly received.

9.4 Discussion

Livestock transporters described a range of factors that had varying impacts on the ease of sheep handling. These fell into the four categories of people, environmental, dog and sheep factors (Figure 9.1). Overall, facilities were believed to have the most impact, followed by sheep experience and breed. Transporters considered patience and experience to be key factors determining how a person might deal with difficult sheep, and described a lack of formal training available to the industry.

9.4.1 People

The impact of stockman (non-gender specific) attitude on handling behaviours has been demonstrated in a variety of livestock species, including pigs (Hemsworth *et al.*, 1989; Coleman *et al.*, 2003), dairy cattle (Hemsworth *et al*., 2000; Lensink *et al*., 2000), beef cattle and sheep (Coleman *et al*., 2012). In sheep, stockmen whose attitudes suggested more awareness of how the immediate surroundings affected the animals tended to use aversive techniques such as shouting less than other handlers.

Those who felt that they were under severe time pressure were more likely to push animals harder using frequent whistling and hits (Coleman *et al*., 2012). This indicates that attitudes towards livestock handling can affect handling behaviours and it is reasonable to imply that this could have flow on effects on ease of handling. Transporters indicated that in general, the people assisting during handling were helpful, having a positive impact on ease of sheep handling, with negative attitudes being less common. The common use of anthropomorphism among the transporters, equating sheep feelings and values with human ones, demonstrated a great deal of empathy and care for the animals. The broad range of factors described by the transporters also indicated that they are very aware of how a wide range of circumstances can affect sheep behaviour and thus their ease of handling.

9.4.2 Environment

Many of the principles of efficient and effective livestock facility design are reported by Grandin (1990). Transporters observations regarding the important aspects of yard design match with what are presented in the literature, and transporters indicated that there is a slow but noticeable change towards better facilities in all sections of the industry (abattoirs, saleyards and farms). However, given the emphasis put on the impact of facilities on ease of handling by transporters and their descriptions of the poor facilities still common on many farms, it appears that this is an area in need of further improvement.

The majority of other environmental factors described by transporters have not been investigated previously. It is unlikely that further research is necessary into the effects of shadows, lighting and noise levels on livestock stress and ease of movement as they have been well described anecdotally and are well accepted by much of the industry and research community (Grandin, 1990; Weeks, 2008). There is little evidence regarding the impacts of time of day and weather, but transporters described these as being of minimal importance, indicating that experimental trials are unnecessary.

9.4.3 Sheep factors

Transporters emphasised the importance of previous experience for sheep, and this is supported by evidence from a number of experimental studies. Sheep that have had positive experiences during handling are easier to move through yards and facilities (Hutson, 1985) and previous exposure to humans decreases fear reactions (Romeyer and Bouissou, 1992; Markowitz *et al.*, 1998; Viérin and Bouissou, 2003; Erhard *et al.*, 2006). Sheep are able to habituate to human handling, and by reducing fearfulness, sheep are more likely to react calmly and consistently to handling, as reported by the transporters interviewed.

In addition to the effects of experience, breed was also an important factor to transporters. Breed differences in fearfulness and reactivity to humans have been demonstrated experimentally (Romeyer and Bouissou, 1992; Le Neindre *et al.*, 1993; Viérin and Bouissou, 2003). Le Neindre *et al*. (1993) reported variation between breeds in ease of movement through facilities, and differences in reaction style to stressors have also been described (Boissy *et al*., 2005). Shedding 'hair' breeds such as the Dorper and Damara have grown in popularity over the last fifteen years due to reduced labour requirements compared to more traditional breeds such as the Merino (Almeida *et al.*, 2013; Alemseged and Hacker, 2014), and quantitative differences in avoidance behaviours between Dorpers and Merinos have been found (Njisane and Muchenje, 2013). Consequently, breed differences in behaviour are likely to become more apparent and more important for efficient handling of sheep as the balance of breeds changes.

Transporters indicated that weakness in sheep, caused by poor preparation, over- or under-fatness and advancing age, had a minor negative impact on the ease of handling. New Australian Animal Welfare Standards for the Land Transport of Livestock were introduced in 2012 which included specific requirements in relation to which animals are fit to load (Anonymous, 2012a). It is likely that this campaign has resulted in fewer sheep in poor condition being transported, which may contribute to the lack of impact of condition on sheep behaviour reported by livestock transporters. Body condition has been shown to affect activity in cattle, with animals in poor condition displaying lower levels of activity than those in good condition (Fordyce and Goddard, 1984), although the effect of over-fatness has not been examined. No such studies appear to have been done in sheep. Likewise, the observation that sheep in full wool are more difficult to move has not been explored previously and these could present a valuable directions for some future research.

Sex differences in the reactivity of sheep have been reported repeatedly (Vandenheede and Bouissou, 1993; Viérin and Bouissou, 2003; Boissy *et al.*, 2005). However, participants in the present study repeatedly indicated that sex was not an important factor in ease of handling sheep. This disparity may be partly due to the infrequency with which the transporters reported transporting rams, but is likely to be primarily a function of the situations in which these sex differences were demonstrated in the previous studies compared to the present one. Arena tests have been the most commonly used test, in which sheep are isolated from flock mates and exposed to additional stressors such as surprises, novel objects and human presence. While many of these types of stressors are present during loading and unloading, sheep are generally handled in flocks and very rarely isolated. When compared to isolated ewes, grouped ewes show far less fear behaviours when presented with these stressors (Kilgour and Szantar-Coddington, 1997).

Transporters described an innate characteristic of sheep as individuals that gave a degree of unpredictability to each handling event, using phrases such as “it comes down to the sheep”. Transporters commonly used anthropomorphism and personification of the sheep, attributing them with human-like personalities and using this to explain the unpredictable nature of sheep. In one instance a transporter specifically said that the differences between sheep that he did not understand were due to differing temperament. The concept of temperament in livestock is not new, and is generally understood to be an animal’s inherent potential to respond in a particular way to a stimulus (Dodd *et al*., 2012). French cattle farmers described temperament as the first trait considered when culling (Boivin *et al*., 2007), illustrating the importance of the individual animal's temperament for ease of handling. A variety of behavioural tests exist to measure behavioural reactivity, the phenotypic expression of temperament, in sheep (for review, see Dodd *et al.*, 2012). These tests reveal variability between animals in their reactions to different stimuli, such as isolation, restraint and novelty, and are generally both repeatable and heritable, demonstrating that individuals behave differently to each other in a given situation, that these differences are consistent over time and have some genetic basis.

9.4.4 Training

The desire for formal stock handling training tailored to the livestock transport industry was an interesting outcome of this study. Stock handling training exists currently in Australia focused on cattle, sheep and pigs on farm, in feedlots and at abattoirs, but such training would not be directly transferrable without modification for the livestock transport industry. This result highlights an area for further development in the industry.

9.4.5 Limitations

This was a small exploratory study based on a limited number of transporters drawn from the membership of the Livestock Transporters Association of South Australia. While the attitudes and experiences of these transporters cannot be said to be representative of livestock transporters across Australia, the speed with which thematic saturation was reached gives the authors confidence that the opinions expressed were genuinely shared by those interviewed. Due to small sample sizes, samples in qualitative research are never representative and are prone to selection bias. While all transporters in this study had been transporting livestock for many years, with the least time transporting reported as “almost ten years”, it was felt that it was important to interview those who were more likely to have extensive experience and established opinions regarding sheep handling and behaviour. All of those interviewed were owner-operators, neither employed by a larger company nor employing anyone else, although some reported employing others previously. It is possible that transporters associated with larger companies may experience sheep behaviour differently and thus express different views of the factors influencing ease of handling.

Interviews were directed to livestock transporters to take advantage of their varied experience in regards to range of sheep handled (age, condition, breed, previous experience) and conditions of handling (weather, yards, availability of helpers). As transporters generally only handle sheep during loading and unloading of trucks, this limited the study in terms of the types of interactions between handlers and sheep. Alternatively, interviews could have been directed to sheep farmers, but it was felt that farmers were more likely to only handle a limited variety of sheep in respect to breeds, condition and experience. Interviewing transporters allowed comparisons to be made between these different types of sheep, but potentially limited the study in respect to observations regarding behaviour of individual sheep (i.e. personality or temperament) as transporters generally only handle any animal once in its lifetime.

A limitation of qualitative research is that it relies on what people say, rather than independent observation of what they actually do. Some respondents may feel the need to 'prove' their expertise by highlighting many factors and placing strong emphasis on factors that may not actually be that important. However, probing questions were repeatedly used during the interviews in an attempt to verify the findings and enhance the credibility of this study. Triangulation resulted in similar strength of statements around each factor both within and across interviews (Patton, 1990).

This study utilised interviews as a method to gain a deeper understanding of the perceived importance of the factors affecting ease of handling, as seen by those working in the industry. Quantitative studies assessing sheep behaviour have generally looked at factors in isolation rather than as a complex, interacting system, and often under non-commercial conditions. Additionally, although previous studies have investigated sheep behaviour, ease of handling has not been investigated and is likely to be difficult to quantify. The use of a qualitative method such as thematic analysis of interviews allowed the authors to explore this relatively new field and describe the relationship between variables as understood by those in the industry.

9.5 Summary

Livestock transporters described a range of factors that had varying impacts on the ease of sheep handling. Overall, facilities were strongly believed to have the greatest impact, followed by sheep experience and breed. Transporters also discussed the effects of distractions, time of day, weather, dogs, other people, sheep preparation, body condition and sex on ease of handling. The concept of individual sheep temperament was indirectly expressed. Transporters considered patience and experience to be key factors determining how a person might deal with difficult sheep and demonstrated their care and professionalism in their attitudes towards sheep.

A number of areas for further research and development were identified. Some participants expressed a desire for formal handling training to be available to the industry, and this could form part of a quality assurance program for livestock welfare. A need for improvement of facilities, particularly on farms, was also described. There is scope for further research to confirm anecdotal evidence regarding the effects of lighting and shadows on sheep behaviour, and additional work could also be done to explore the impacts of body condition and wooliness on behaviour of sheep.

Chapter 10. Impact and conclusions

Sheep at Struan Research Centre during behavioural testing, December 2011.

10.1 Introduction

The ability to measure behaviour allows researchers to investigate the relationships between temperament, stress and productivity in livestock. Subsequently this information can be utilised in improving livestock production systems, minimising stress and maximising ease of handling and efficiency of production. The aims of this research were threefold: initially to assess a range of measures of behavioural reactivity in sheep to identify one or more that were suitable for use in large research or commercial contexts; secondly to assess the effect of frequency of handling on behavioural reactivity; and thirdly to establish the importance of behavioural reactivity by assessing its relationship to carcass composition and meat quality traits.

The first aim to assess a range of measures of behavioural reactivity was achieved. After review of the literature, a set of behavioural tests which met the criteria of being relatively simple to incorporate into normal management procedures were selected for further investigation. Analysis of the Information Nucleus flock dataset (Chapters 4 and 5) yielded heritability estimates for two of the behavioural tests, and further trials established the repeatability of each test (Chapters 3 and 6). The use of salivary cortisol at behavioural testing (Chapter 6) and exsanguination blood cortisol, lactate and glucose at slaughter (Chapter 8) allowed the relationships between reactivity and stress to be demonstrated. However, these point measurements did not have sufficient power to establish clear links between individual behavioural tests and stress. This research successfully demonstrated associations between behavioural reactivity on farm and meat quality (Chapter 8), but was unable to demonstrate these same relationships in the larger and more complex Information Nucleus dataset (Chapter 5), for reasons described below.

This body of work also aimed to assess the effect of frequency of handling on sheep behavioural reactivity. This aim was achieved, concluding that in this respect, research sheep are representative of commercial sheep, as described below. The impacts of other variables, such as sex, breed, previous experience and facilities, on behaviour of sheep was also investigated by both experimental trial (Chapters 2-8) and through interviews of livestock transporters (Chapter 9), using triangulation of quantitative and qualitative methods across the thesis to give strength to the conclusions drawn (Patton, 1990).

10.2 Support for the concept of temperament and behavioural reactivity

During interviews of livestock transporters, the concept of individual sheep temperament was described using phrases such as "*It all depends on the animal itself*" and by attributing individual personalities to sheep (Chapter 9.3.4.6). The initial working concept of temperament derived from the literature states that temperament is an animal's inherent potential to respond in a particular way to a stimulus, and is determined by genetic and permanent environmental effects such as early life experience (Chapter 2). This inherent temperament does not change over time, but its expression is influenced by the animal's temporary environment. The measurable expression of temperament is behavioural reactivity (Bates *et al.*, 1995; Burrow, 1997;

Viérin and Bouissou, 2001). Throughout this research, evidence was collected to examine the concept of temperament and the phenomenon of behavioural reactivity.

To establish the phenomenon of behavioural reactivity, it was necessary to demonstrate repeatable differences between sheep in the way they responded to stimuli, and to determine the relationship between these behaviours and stress. Review of the literature indicated that both restrained and unrestrained tests were generally moderately to highly repeatable (Murphy *et al.*, 1994; Kilgour and Szantar-Coddington, 1995; Wolf *et al.*, 2008) with the exception of objective flight speed which had a reported repeatability of as low as 0.008 which is equivalent to random (Blache and Ferguson, 2005). In the flight speed trial (Chapter 3), repeatability was estimated to be between 0.52 and 0.59 across days one week apart, indicating that the low values reported by Blache and Ferguson (2005) were likely a result of their protocol, rather than problems with the flight speed test itself. Correlations between repeated measures of a variety of behaviours up to 175 days apart in the Struan trial (Table 6.2) were generally moderate to strong, supporting the hypothesis that behaviour of sheep is repeatable.

In addition to repeatability of behaviour, it was important to demonstrate differences in reactivity between sheep. Behaviours measured in the Struan trial had high coefficients of variation (CV) of between 0.42 and 0.88 (Chapter 6). Similarly, flight speed and agitation in the Information Nucleus had CVs of 0.50 and 0.68 respectively (Chapter 5). Variance in the flight speed trial was smaller (CV = 0.20-0.24), this likely reflected technical random error in the Information Nucleus due to the large number of people collecting data. The flight speed trial showed that a large portion of the variation in flight speed was between-sheep (repeatable) rather than within-sheep (Figure 3.2). Repeatable variance in flight speed ranged between $0.18m^2/s^2$ and $0.31m^2/s^2$ (standard deviations of 0.43-0.55m/s) in both the flight speed and Struan trials, indicating that regardless of technical error or differences in both context and genetics between the trials, the amount of variation in flight speed between sheep remained constant. These results demonstrate that individual sheep differ markedly and reliably in their responses to stimuli.

As well as establishing that measures of behavioural reactivity in sheep are repeatable and vary between animals, it was also necessary to establish a link between these behaviours and physiological indices of stress. Physiological measures, comprising heart rate and salivary cortisol at behavioural testing and plasma cortisol, lactate and glucose at slaughter, were measured in the Struan trial (Chapters 6 and 8). Temperament (PC1) was negatively associated with basal salivary cortisol during behavioural testing, suggesting that more reactive lambs may have been experiencing chronic stress (Visser *et al.*, 2008; Duan *et al.*, 2013). Lambs that were more reactive during behavioural testing also appeared to experience acute stress at handling immediately prior to slaughter, indicated by elevated plasma cortisol at exsanguination. These results were supported by a trend towards higher lactate in lambs with lower PC2 scores (faster flight speeds, less reactive in restrained tests) and by associated effects on loin pH of PC1 and plasma glucose and lactate, whereby both more reactive lambs and lambs with elevated glucose and lactate developed lower meat pH after slaughter. This relationship between reactivity and stress metabolites indicates that behavioural reactivity tests actually do reflect neuro-endocrine responses to stimuli and thus that behavioural reactivity is likely to be a true representation of the stress response of individual sheep. However, further studies specifically designed to assess physiological responses to stress with sheep sampled at the same time points rather than at a range of time points during behaviour tests are necessary to confirm this relationship.

As stated above, temperament was defined as an animal's inherent potential to respond in a particular way to a stimulus, determined by genetic and permanent environmental effects. To test this theory, it was necessary to determine how much of the variance in the behaviours measured was attributable to genetics and permanent environment. Very few estimates of heritability were available in the literature (Chapter 2), but those reported indicated moderate heritability for most traits (Blache and Ferguson, 2005; Wolf *et al.*, 2008; Plush *et al.*, 2011). Further evidence for a genetic influence was found, with different breeds of sheep displaying different reactivity within trials, and cross-bred sheep having reactivity intermediate to the two parental breeds (Le Neindre *et al.*, 1993). Successful divergent selection for sheep based on measures of reactivity (agitation during social isolation and reaction to a human in an arena) indicated that the trait is at least partly heritable (Blache and Ferguson, 2005; Beausoleil *et al.*, 2008; Amdi *et al.*, 2010).

As hypothesised, breed differences were seen in both the Information Nucleus and the Struan trials. First cross Terminal Merino lambs were more reactive than the other breeds (Chapter 4, Table 5.3, Table 6.3). This similarity in breed effects between trials was expected, as there were shared genetics between the lambs in the two trials, but this result lends support to the concept of a genetic component to behavioural reactivity. Breed effects were cited by livestock transporters as one of the most important factors influencing sheep behaviour (Chapter 9), although this was between cleanskin (Dorper, Damara) and wooled-type breeds (purebred and crossbred Merino). Crossbred Merinos were perceived by livestock transporters to be behaviourally indistinguishable from pure Merinos. Any differences between Merinos and their crosses are likely to small compared to the large differences reported between cleanskin and wooled breeds. There were breed differences in plasma cortisol at slaughter, with 1st cross lambs having much higher cortisol at slaughter (63.9nM ± 6.4) than 2nd cross (39.4nM ± 8.6, P=0.029, Chapter 8), indicating that there are real differences in stress response between breeds.

Analysis of the Information Nucleus dataset resulted in low to moderate heritability estimates for flight speed (0.11±0.02) and agitation (0.19±0.02, Table 4.1), consistent with previous estimates for these tests of behavioural reactivity. This provides evidence that genetic or permanent environmental differences in behavioural reactivity between sheep exist, not only between breeds, but also within them, although these genetic effects are small. The small proportion of behavioural variance accounted for by the maternal permanent environmental effect in the Information Nucleus (Chapter 5) and the effect of lambing paddock on behaviour at weaning but not later in life (Chapter 6) suggest that the heritable between- and within-breed effects are primarily genetic rather than due to permanent environmental effects such as early life experience. Together these results verify that a significant component of behavioural reactivity is determined by genetics, supporting the concept of inherent temperament of sheep.

In summary, this thesis has divided behavioural reactivity into its components, allowing this complex phenomenon to be better understood (Figure 10.1). As evidenced by moderate heritability estimates (Chapters 4 and 5) and the importance of breed (Chapters 4, 5 and 6), there is an important additive genetic component to behavioural reactivity. This additive genetic effect, in addition to what are likely to be small dominance and epistatic effects, comprise the genetic component of behavioural reactivity, estimated to account for approximately 17% of the total variance in behaviour. In the Information Nucleus it was demonstrated that the maternal effect (including maternal environment) was small (Chapters 4 and 5), thus the majority of inherent sheep temperament is additive genetic. In addition to inherent temperament, the repeatable portion of behavioural reactivity includes a large permanent environmental effect, approximately 50% of total variance in behaviour. This is responsible for the sizable difference between estimates of repeatability and heritability found throughout this thesis. The majority of this permanent environmental effect is likely to be sheep experience; this is discussed in further detail in section 10.3. The effect of the interaction of genetics and environment on behaviour was demonstrated to be small in the Information Nucleus (Chapters 4 and 5). Context, such as physiological status of the sheep (Chapter 2.4.5) and the facilities used for testing (Chapters 6.4 and 9.4.2) has a moderate impact (15% of variance) on behavioural reactivity. Finally, a moderate proportion of the variance in behavioural reactivity (15% of variance) is attributable to temporary environmental effects, including technical error.

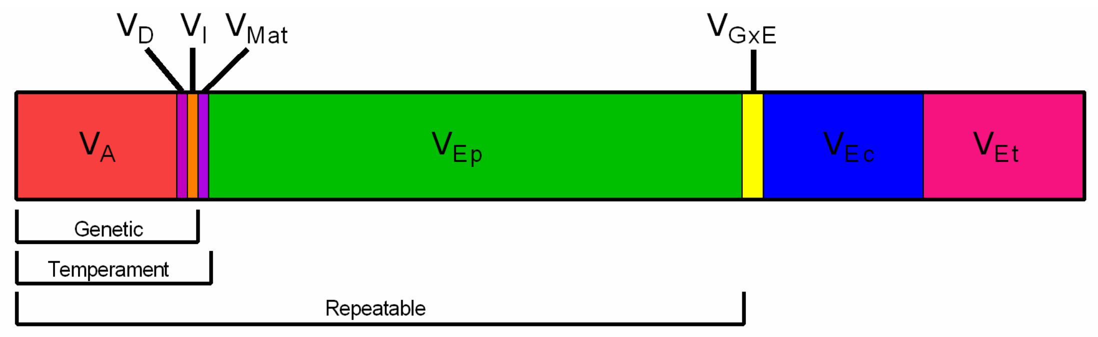


Figure 10.1. The variance components of behavioural reactivity. V_A = additive genetic, V_D = dominant genetic, V_I = epistatic, V_{Mat} = maternal, V_{Ep} = permanent environmental, V_{GxE} = interaction of genetics and environment, V_{Ec} = environmental (context), V_{Et} = environmental (temporary).

10.3 Age and experience

Sheep experience was rated as very important by livestock transporters, who reported that sheep that had been handled, preferably trucked, previously were much easier to handle compared to unhandled, naïve sheep, and that sheep accustomed to poor facilities became more difficult to handle over time (Chapter 9). This agrees with reports in the literature (Chapter 2.4.3) that indicate that as lambs habituate to handling, reactivity is reduced (Hutson, 1985; Hargreaves and Hutson, 1990b; Erhard *et al.*, 2006).

Age and experience, although confounded, also appeared to be important in the Information Nucleus analysis and the flight speed and Struan trials. In the flight speed trial, lambs were faster on Day 1 compared to Day 2, suggesting that they had habituated to some extent in the week intervening (Table 3.1). In the Information Nucleus dataset there was an effect of age on the outcome of the agitation test, such that older lambs in one flock were less reactive in the agitation test (Chapters 4 and 5).

Lambs in the Information Nucleus were only tested once, so this is likely due to habituation to humans rather than to the experimental protocol. The Struan trial highlighted differences due to age at testing, with PC3 distinguishing behaviour at weaning, when the lambs were naïve to the protocol and had been handled relatively few times, from behaviour at post-weaning and pre-slaughter, when it may be expected that the lambs had habituated to some extent (Table 6.3). Loadings on PC1 indicated that behaviour at weaning was less important than behaviour later in life in predicting overall reactivity. In humans, behavioural consistency improves with age, probably as a result of accumulated experience with the environment (Roberts and DelVecchio, 2000). That this is also the case in sheep is supported by evidence of greater repeatability of behaviour in older animals and poor repeatability for animals that were naïve to the testing protocol (Horton *et al.*, 2009). This may explain the lack of relationships found between behaviour and carcass quality in the Information Nucleus, where lambs were only behaviourally measured at weaning.

10.4 Flight speed and restrained tests measure different aspects of temperament

The literature review described three types of behavioural tests: unrestrained, restrained and maternal (Chapter 2). Of interest for this research were those that could be integrated into routine management of sheep. Few estimates of correlations between such behaviours had been made and these indicated that flight speed was positively but weakly correlated with restrained behavioural measures such as agitation or the subjective crate score (Blache and Ferguson, 2005; Pajor *et al.*, 2008; Hocking Edwards *et al.*, 2011; Plush *et al.*, 2011). Similar results were found in the analysis of the Information Nucleus data, which also failed to find a strong relationship between the two types of behavioural test (Tables 4.2 and 5.4). When explored in the Struan trial, although all of the behaviours investigated had positive loading on PC1 (29% of variance) indicating that they contributed to temperament, the restrained behaviours (agitation, subjective crate score and CV of weight) had opposing loadings on PC2 (14% of variance) compared to the two measures of flight speed. This indicates that flight speed measures a distinctly different component of temperament than do restrained behavioural tests. This understanding is valuable; it may be that particular aspects of behaviour are more likely to be related to production traits, and it is important to get a complete picture of behavioural reactivity if there is to be selection of animals based on reactivity.

10.5 Females are more reactive than males

A consistent finding in the literature review and, as hypothesised, the experimental chapters was greater reactivity in ewes compared to wethers. The literature review presented articles reporting greater expression of fear behaviours in ewes and higher cortisol responses to isolation and ACTH administration (Vandenheede and Bouissou, 1993; van Lier *et al.*, 2003; Viérin and Bouissou, 2003; Boissy *et al.*, 2005; Hernandez *et al.*, 2010). This supports the results of all of the experimental chapters, where females were more reactive, regardless of the test used, than males (Tables 3.1 and 6.3, Chapters 4 and 5).

In contrast to the behaviour traits measured on individual sheep, livestock transporters indicated that sex was of minimal importance and ewes and wethers were behaviourally indistinguishable when handled as mobs (Chapter 9). This discrepancy is likely due to context. During behavioural testing lambs are often physically, if not visually, isolated from flock mates. Studies in cattle suggest that females are much more socially motivated than males (Boissy and Bouissou, 1995), and thus it stands to reason that they would be more behaviourally agitated during isolation. However, livestock transporters would rarely attempt to handle isolated sheep, instead working flocks of sheep as they are loaded and unloaded from trucks. Since the differences seen between ewes and wethers in experimental studies are most likely generated by social isolation, there is no reason to expect ewe lambs to be more reactive than wethers during flock handling, thus the lack of perceived importance to livestock transporters.

10.6 Behavioural reactivity is related to meat quality as indicated by loin pH

In contrast to the hypothesis that behaviours and carcass traits would be associated, few phenotypic or genetic relationships were found in the analysis of the Information Nucleus data (Tables 5.6 and 5.7). However, in the Struan trial behavioural reactivity was related to aspects of carcass quality, albeit opposite to the relationship expected, with higher reactivity being associated with better loin pH. Lambs that had more reactive temperaments (PC1) appeared to be stressed in lairage, most likely as they were moved to the stunning area, triggering lactic acid production. Since this occurred immediately prior to slaughter, the lactic acid may not have been cleared from the muscles by the circulatory system, resulting in a longer duration of lactic acid production and subsequently lower loin pH for these lambs compared to those with low overall reactivity. A similar result has been seen previously in Romane sheep slaughtered at approximately 126 days of age (approx. 184 days younger than the lambs in the Struan trial), although no attempt was made to explain the negative correlation between stress and ultimate pH (Deiss *et al.*, 2009). Psychological stress immediately prior to slaughter has been demonstrated to accelerate pH decline in pigs (D'Souza *et al.*, 1998), thus by the time the carcass is measured at approximately 24 hours post-mortem, lactic acid production has been occurring for longer than in the unstressed animal. In conclusion, although the majority of carcass traits do not appear to be related to behavioural reactivity in sheep, reactivity on the farm is associated with the stress reaction to peri-mortem handling and can affect post-mortem muscle pH.

10.7 Management group affects reactivity

Although not apparent from the literature (Chapter 2), the results of the Information Nucleus analysis, flight speed trial and Struan trial indicate that there are important management group effects, not only on behaviour itself but on its relationship with other variables such as carcass traits. A total of 96 management groups were represented in the Information Nucleus, and the differences between groups accounted for approximately 20% of the total variance in each behaviour (Tables 4.2 and 5.5). Differences in flight speed were measured between pens of lambs in the flight speed trial (Table 3.1), and lambing paddock affected behaviour at weaning in the Struan trial indicating that differences in early life experiences between groups influenced later behaviour (Chapter 6).

Significant phenotypic relationships were present between behaviour and loin pH 24 hours post-mortem in the Struan trial (Table 8.2), but this was not observed in the much larger Information Nucleus flock (Tables 5.6 and 5.7). It is possible that this is due to management group differences in the relationship between behaviour and pH; i.e. some management groups may have had a strong relationship between the two traits while others had weak or no relationship. To test this, a further model was fitted to the INF data. Fixed effects of flock, lamb age (nested within flock), sex, flight speed (natural log transformed as described in Chapters 4 and 5), management group (within flock and year), and the interaction of flight speed and management group were fitted to loin pH 24 hours post-mortem. This model allowed the comparison of the overall effect of flight speed to the within-management group effect of flight speed. Even when the interaction was significant, examination of individual slopes representing the relationship between flight speed and loin pH within a management group indicated that none were significantly different from zero. When agitation was also re-assessed in this way, the same result was found. This indicates that it is unlikely that management group differences were the cause of the lack of relationships found between behaviour and carcass quality in the Information Nucleus. This leads to the hypothesis that the young age of assessment and thus lack of experience in the lambs of the Information Nucleus was the most likely cause.

Livestock transporters emphasised the impact of facilities on sheep behaviour (Chapter 9) and this factor may have impacted on the management group effects seen between Information Nucleus sites. Other factors such as maternal environment (Chapter 2.4.4), flock health and nutrition (Chapter 2.4.5) and differences in rainfall and temperature between sites (Chapter 5.4) could also have contributed to management group effects on behaviour.

10.8 Methodological advances

Three principal methodological advances were made during this research. The first of these was in regards to the measurement of flight speed. In contrast to the hypothesis that a shorter distance would be more appropriate for measuring flight speed in sheep (compared to the distance for cattle), 1.7 and 2.0m were more repeatable than shorter distances, had the most variance between sheep and were able to detect differences between sheep based on pen, day and sex (Chapter 3). The measurement distance for flight speed in sheep had not previously been validated, and as such this is a valuable contribution to the field.

The usefulness of face cover score and hairline position as indicators for a variety of measures of behavioural reactivity were also assessed. While all three measures of facial hair patterning (subjective face cover score, ratio hair line position and ordinal hair line position) were moderately repeatable, none were related to the three principal components describing behaviour (Chapter 7). This contrasts to results in other species, where hair whorl position is predictive of behavioural reactivity. While the mechanism for the relationship between hair whorl position and behaviour has not been confirmed, it is likely that these disparate results are due to differing pre-natal origins since hair whorl position is a measure of hair patterning (direction) whereas face cover and hair line positions describe regional differences in pelage. Again, this work had not previously been done in sheep and these results give strong evidence against the future use of facial hair patterning as an indicator for behaviour in this species.

One of the objectives of this research was to assess whether behavioural studies conducted in the Cooperative Research Centre for Sheep Industry Innovation's "Information Nucleus" flocks could be generalised to the commercial population of sheep given the high frequency of handling that occurs in these research flocks. The lack of significant handling effect (Chapter 6) suggests that the behaviour of "research flock" lambs in previous studies (Lennon *et al.*, 2009; Hocking Edwards *et al.*, 2011; Plush *et al.*, 2011; Dodd *et al.*, 2013; 2014) is representative of similar sheep in a commercial setting; the increase in frequency of handling was insufficient to create behavioural differences in these sheep.

10.9 Theoretical and practical implications

This research has demonstrated that sheep behaviour during handling can be indicative of chronic stress on farm, and predictive of the stress response at slaughter. Sheep that were more reactive on farm had lower basal salivary cortisol, possibly indicating desensitisation of the HPA axis due to chronic stress (Visser *et al.*, 2008; Duan *et al.*, 2013), and went on to have a greater stress response to handling at slaughter as indicated by exsanguination blood cortisol, lactate and glucose. This increased stress response resulted in lower loin pH 24 hours post mortem. On a lower plane of nutrition or after a longer period of lairage more reactive sheep risk exhausting their stores of muscle glycogen. This would result in higher than acceptable ultimate meat pH, leading to toughness, poor meat quality and reduced shelf life (Ferguson *et al.*, 2001; Thompson, 2002). These results show that temperament measured on farm, combining flight speed and restrained tests and measured later in life (after weaning), can be used to predict the reaction of the sheep at slaughter. If this can be confirmed in a variety of flocks, it may be possible to develop a behavioural threshold to determine which sheep are likely to have a sufficiently severe reaction to slaughter that meat quality would be affected. This has real implications for the management of sheep stress on farm and at slaughter, with the potential to improve both meat quality and the welfare of the sheep.

This thesis demonstrated a component of behaviour that was consistent across traits and heritable (temperament), which would suggest that it is possible to genetically select sheep for lower reactivity. However, heritability estimates were moderate at best and with limited knowledge of the genetic relationships between behavioural reactivity and many other economically important traits, such as wool production (Plush *et al.*, 2011) and feed efficiency (Amdi *et al.*, 2010), it would be unwise at this point to select based on reactivity.

While these studies show that reactivity may be related to stress, further work is needed to establish the links between individual behavioural tests and stress. Point measures of salivary cortisol and heart rate may be insufficient to achieve this; future work should also investigate alternative physiological stress indicators such as heart rate variability (Pagani *et al.*, 1991), chromogranin A (Escribano *et al.*, 2014), and αamylase (Matsuura *et al.*, 2012). Greater understanding of the relationships between the behavioural and physiological responses to stress will improve both farm productivity and animal welfare.

Appendix 1. Flight speed measurement

1. Release sheep from weigh crate.
2. Video record the movement of the sheep past the striped scale as shown below, using a video camera set to 50 frames per second.
3. Break video footage into individual frames, each 0.02 seconds apart.
4. For each frame including and after the first frame in which the sheep is in front of the scale, record the position of the most advanced part of the sheep in respect to the scale.

Appendix 2. Face cover scoring

Age: Over 4 months, with minimum of 4 months wool.

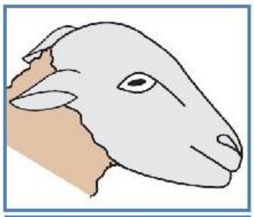
Face cover refers to the degree of wool cover on the face, including the top of head and jowl.

How to score: A single score of 1, 2, 3, 4 or 5.

Rule of thumb: An animal with Score 1 has an open face with no wool on the jowls or top of the head. A Score 5 animal has wool covering its entire face, commonly referred to as 'wool blind'.

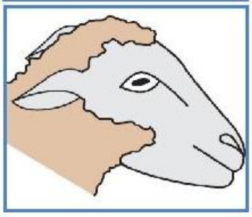
Score 1:

Open face with no wool in front of the ears and topknot, or on the jowls.



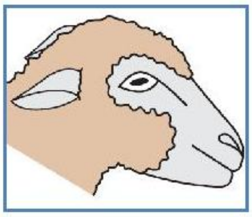
Score 2:

Wool cover over the top of head; some on the side of muzzle, but not joined between the ears and eyes.



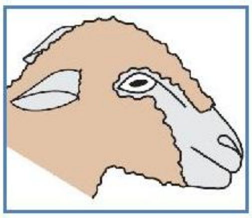
Score 3:

Wool cover over the top of head and on the side of muzzle; wool joined between the ears and eyes.



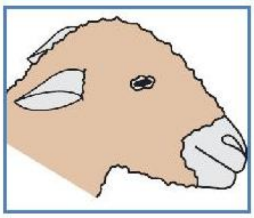
Score 4:

Wool cover from the top of the head down the muzzle; clear channel remains between the eye and the mouth.



Score 5:

Heavy wool growth over the entire face; wool from the top and side of the muzzle joining.



Appendix 3. Interview schedule

Experience/training/demographics

Can you describe your business to me? How many people, family/employees, species, trucks, decks?

Tell me about your experience trucking livestock. How long have you been transporting animals?

Have you always transported stock or have you been driving trucks for longer?

Where did you get most of your stock handling experience? How do you feel about what you were taught? What were the best things? What didn't work? Do you find sheep handling easy or hard? Compared to other species?

How many loads of livestock would you transport per week on average? How many of those would be sheep?

Where do you transport from and to?

Over what kind of distances do you transport stock?

Do you always do the same runs? How often do you go to new places?

Take me through your typical work day.

What's your biggest stress?

Loading/unloading

What affects how easy it is to load sheep onto a truck?

What about:

- Weather
- Facilities
- Time of day
- Breed
- Lambs
- Older sheep
- Sex (rams vs. females vs. wethers)
- Curfew
- Use of prodders – do you have them?
- Dogs – difference in how sheep react?
- Helpers

Is unloading any easier or harder than loading, generally? Why?

What have you seen other drivers do with really difficult sheep?

Do you ever see other drivers lose their temper with sheep? How common is this? What happens?

If there were one thing you could change to make sheep handling easier, what would it be?

Do you get any feedback on your sheep handling from anyone? What kind of feedback – eg time, bruising?

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