Improving beef quality through pre-slaughter cattle management

This thesis is presented for the degree of

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by

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THE UNIVERSITY of ADELAIDE

This thesis is dedicated to the steer;

Overlooked in the paddock, unsurpassed on the plate.

To wash, or not to wash? That is the question –

To wash, perchance to clean – ay, there's the rub,

For in that wash of water what clean may come?

| Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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July 2021

| Abstract

Australian beef is renowned the world over for its high quality and safe production in a clean, green environment. One of the greatest contributors restricting the supply of quality product is dark cutting beef. While this quality defect has been the centre of much research, there is a comparatively small understanding of the importance of lairage factors and their impact on this problem. Given time spent in lairage is an inevitable component of beef production, the aim of this project was to understand the role of pre-slaughter factors, specifically dark cutting and pre-slaughter hide washing practices. This work, conducted at one large southern Australian processor, included an observational cohort study of 3,054 cattle and historical data analysis using 2,929 records.

A literature review identified a paucity of information on pre-slaughter hide washing, a common practice in Australian abattoirs where live animals are cleaned to remove mud, faeces and bedding from the hide. Australian and International Standards require cattle to be washed prior to slaughter to ensure they are in a clean condition for processing. However, the effectiveness of this procedure at controlling hide and carcase microbial contamination is questionable. Additionally, it has been shown to negatively affect meat quality and may adversely affect welfare.

Lairage factors affecting dark cutting incidence and animal behaviour were identified and spanned from the time of unloading until the point of slaughter. Behaviours observed during unloading (jumping, mounting, exit score, unloading time, number of stock on truck) and in lairage (head down) were associated with an increase of up to 18.5% and 0.3%, respectively, in dark cutting incidence. Pre-slaughter hide washing was also associated with an

increase in dark cutting incidence of 5.5% for each additional wash animals received while in lairage pens. The time of day, and number and duration of washes with a high-pressure hose also had a significant effect on behaviour. A more targeted study described the change in behaviour resulting from washing, including an increase in behaviours indicative of stress and a decrease in resting behaviours. Beef carcase hygiene records were used to identify periods of risk for increased microbial contamination to guide future work.

Current pre-slaughter hide washing practices lack scientific grounding, contribute to increased dark cutting, and change animal behaviour. A recommendation is given for future work to investigate washing with a multi-factorial approach, ensuring beef quality while maintaining safety and animal welfare throughout production.

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of money, we learnt how to get by with what we had, which often required us to be inventive and find solutions outside the box. This life taught me how to think and problem solve in ways that a classroom cannot, and it is these skills learnt in life that have enabled me to succeed in my education, especially this PhD. It has been an absolute privilege being the first person in our family to finish high school, to study at university, and, universe willing, become a Doctor. I feel blessed to have this honour, which is only possible because of the sacrifices, support, and endless love you have given me.

| Publications

Conference proceedings:

Preston, FL, Wilkes, MJ, McGilchrist, P & Pitchford, WS. Cleaning up our cattle: Shining new light on an old practice. Oral Presentation. *Australian Association of Animal Sciences*, 2021, Freemantle, Australia.

Preston, FL, Wilkes, MJ, McGilchrist, P & Pitchford, WS. How’s it hanging? Head down behaviour in beef cattle increases in response to pre-slaughter hide washing and is associated with increased dark cutting incidence. Oral presentation. *International Congress of Meat Science and Technology*, 2019, Potsdam, Germany.

Preston, FL. Pre-slaughter hide washing: What is it? What do we know about it? What’s next? Oral presentation. *Meat Inspection and Quality Assurance Conference*, 2019, Surfers Paradise, Australia.

Preston, FL, Wilkes, MJ, McGilchrist, P & Pitchford, WS. Pre-slaughter hide washing increases dark cutting incidence in pasture finished cattle. Oral presentation. *International Congress of Meat Science and Technology*, 2018, Melbourne, Australia.

Preston, FL, Wilkes, MJ, McGilchrist, P & Pitchford, WS. Pre-slaughter hide washing may be ineffective at improving beef hygiene and negatively affect meat quality and animal welfare: A review. Oral Presentation. *Australian Society of Animal Production*, 2018, Wagga Wagga, Australia.

| Abbreviations

ACTH	Adrenocorticotropic hormone
AUD	Australian dollar
CFU	Colony Forming Unit
ESAM	*E. coli* and *Salmonella* Monitoring program
HPA	Hypothalamic-Pituitary-Adrenal axis
HSCW	Hot Standard Carcase Weight
MSA	Meat Standards Australia
NCMMP	National Carcase Microbiology Monitoring Program
pH_u	Ultimate pH
SPC	Standard Plate Count

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Chapter 1 | Introduction

1.1 Thesis context

The Australian beef industry was worth an estimated AUD$20.2 billion during 2019 based on off-farm meat value (Meat and Livestock Australia 2020). Despite holding only 2% of the global cattle population, Australia produces 2.4 million tonnes carcase weight of beef and veal annually, of which 76% is exported. This places Australia as the world's second largest exporter of beef, with China (27%), Japan (23%), and the United States (22%) representing the top importing markets by volume (Meat and Livestock Australia 2020). Australia is well placed to supply the global beef market with a small population by global standards, coupled with proven beef production capability, resulting in a strong position as net exporters. However, to remain competitive in the international market, we must maintain consistent supply of high-quality Australian beef valued by our trade partners.

Australia's beef grading system is unique with the ability to provide an eating quality guarantee, in addition to traditional carcase yield assessment, thus ensuring customer satisfaction and increasing repeat purchasing decisions (Polkinghorne and Thompson 2010). Meat Standards Australia (MSA) is a quality assurance system which utilises Critical Control Points throughout the supply chain from production through to value add, culminating in a prediction of beef palatability and guarantee of eating quality that is directly linked to consumer satisfaction (Bonny *et al.* 2018). These Critical Control Points form the MSA prediction model and determine eligibility for carcases to be graded, along with calculation of an eating quality prediction for 135 different cut by cook methods (Polkinghorne *et al.* 2008). Overall carcase eating quality can be estimated using the MSA Index, which provides a simplified representation of MSA eating quality predictions and has been a useful tool for benchmarking and producer feedback (McGilchrist *et al.* 2019). Carcases that meet requirements for MSA grading receive a score for each of the 135 cut by cook method combinations, ranging from 3 star (“good everyday”) to 5 star (“premium quality”) products (Bonny *et al.* 2018). This ability to predict and differentiate eating quality is used to underpin brands and has enabled a more appropriate supply of Australian beef to trade partners, aligned with consumer willingness to pay, and resulted in premiums of up to AUD$66 per head for MSA compliant carcases (Bonny *et al.* 2018).

Meat quality has traditionally referred to the intrinsic properties of meat, including those assessed by MSA, related to sensory perception and safety. However, extrinsic properties have become increasingly important to consumers, with such properties centred around well-being of the animal (animal welfare), the consumer (nutritional value), and society (ecological sustainability) (Purslow 2017). Modern consumers are increasingly interested in the production of their food with most willing to pay more for products labelled for their animal welfare and environmentally friendly attributes (Sonoda *et al.* 2018). The opportunity for Australia to provide such products and further differentiate in the global market is apparent with a world-leading eating quality assurance system, high animal welfare regulated by national standards (Animal Health Australia 2016), and the capability to incorporate food safety, hygiene and traceability guarantees (Red Meat Integrity System).

1.2 Motivation for work in thesis

The greatest challenges to Australia's supply of high-quality beef to the global market come from non-tariff trade barriers. International market access requirements include regulations on beef hygiene and animal welfare, in addition to eating quality expectations. Whilst MSA can predict beef palatability, not all carcases presented for grading meet the minimum requirements and thus are ineligible to be sold with this eating quality guarantee due to defects, limiting beef supply and value.

The most significant contributor to non-compliance for MSA grading is dark cutting beef, a condition characterised by a pH greater than 5.71 at the time of carcase grading (Meat and Livestock Australia 2019). Carcases with a high pH are ineligible to receive a MSA grade and any associated quality premiums. Additionally, dark cutting is estimated to result in penalties of $0.59 per kilogram of Hot Standard Carcase Weight (HSCW) to the producer, along with similar losses to the processor (Jose *et al.* 2015). Dark cutting results from insufficient muscle glycogen levels which limit post-mortem muscle acidification. The concentration of muscle glycogen at slaughter is a result of on-farm glycogen stores, impacted by factors such as nutrition, minus any depletion that occurs during the pre-slaughter period from the time animals leave the farm until the point of stunning (Gardner *et al.* 2014).

Dark cutting rates are considerably higher in pasture-finished cattle compared to grain finished cattle (Meat and Livestock Australia 2019). On-farm nutrition is an important contributor to muscle glycogen stores with the extent of glycogen synthesis being an outcome of dietary metabolisable energy intake (Gardner *et al.* 2014). Grain diets are higher in metabolisable energy compared to pasture diets with the potential for the resulting muscle glycogen concentration of grain finished cattle to be more than double that of pasture finished cattle (Tudor *et al.* 1996; Knee *et al.* 2007). A carcase pH greater than 5.5 is likely to occur when muscle glycogen concentration falls below 57 μmol/g (Tarrant 1989), placing pasture-finished cattle, with a lower resting muscle glycogen concentration, closer to this threshold initially and thus more susceptible dark cutting. The higher resting muscle glycogen concentration of grain finished cattle provides a buffer, and whilst not preventing dark cutting, makes it less likely to occur under the same conditions compared to pasture-finished cattle given the greater amount of muscle glycogen that can be mobilised before the concentration critical to dark cutting is reached.

Equally as important as initial muscle glycogen concentration is any mobilisation of glycogen stores from the time an animal leaves the farm until the point of slaughter. Many stimuli and events during the pre-slaughter period have the potential to be perceived by animals as stressors which can activate the fight or flight response (Ferguson and Warner 2008), resulting in the release of adrenaline and acceleration of muscle glycogen metabolism (Ferguson and Gerrard 2014). When kept off feed, as is standard practice in abattoir lairage, glycogen can be mobilised at rates up to 11 μmol/g/hr when cattle are stressed (Tarrant 1989). In comparison, glycogen repletion occurs at only 0.06 μmol/g/hr under fasting conditions (Tarrant 1989). Therefore, there is insufficient time to replenish any glycogen lost due to stress during the pre-slaughter period, making identification and management of pre-slaughter stressors paramount in preventing dark cutting beef.

In Australia, cattle are washed prior to slaughter to remove contaminants from the hide and comply with national and international standards for food safety (Australia and New Zealand Food Regulation Ministerial Council 2007). Whilst limited studies have examined the relationship between pre-slaughter hide washing and dark cutting, they have demonstrated the potential for this process to be perceived as a stressor, activating the stress response and initiating glycogen breakdown with the potential to contribute to dark cutting beef (Ferguson and Warner 2008). Although pre-slaughter washing is intended to improve beef hygiene, the evidence to support the efficacy of this is limited. Furthermore, the perception of pre-slaughter washing treatments as stressors by cattle, activating the fight or flight response, suggests animal welfare is adversely affected by this practice. Thus, there is considerable potential for pre-slaughter hide washing practices to impact both intrinsic and extrinsic meat quality, limiting value and supply of high-quality Australian beef.

Throughout this thesis, 'overall meat/beef quality' will be used to refer to the combination of both intrinsic (factors that can be determined from the product) and extrinsic (factors associated with the way product is produced) meat quality attributes (Hocquette *et al.* 2014). Of particular importance to this work in reference to overall meat quality is dark cutting beef, meat hygiene, and animal welfare. Dark cutting, and prevention of its development, is particularly important to intrinsic meat quality with a pH less than 5.71 generally providing favourable outcomes for other intrinsic quality attributes. Meat hygiene is regularly monitored and assessed by evaluating levels of microbial contamination on the carcase to ensure food safety for consumers and meet eligibility requirements for trade. Animal welfare exists on a spectrum and can be assessed using many methods, including non-invasive techniques such as observation of animal behaviour. Animal behaviours can thus be used to identify stressors which can then be managed to prevent adverse welfare and contribute to continual improvement of animal wellbeing.

1.3 Thesis aims

The overarching goal of the work described in this thesis is to provide a greater understanding of pre-slaughter factors affecting overall beef quality, with a specific focus on pre-slaughter hide washing. The work in this thesis initially aimed to: 1) identify environmental factors and animal behaviours from the pre-slaughter period (unloading, lairage holding yards, pre-slaughter hide washing) associated with dark cutting incidence; and 2) identify environmental factors which impact animal behaviour during the pre-slaughter period. Following commencement of data collection for this work, another aim was developed to: 3) understand the effect of pre-slaughter hide washing in lairage on animal behaviour. The cumulative results of this work established a need to understand the effect of pre-slaughter hide washing with a multi-factorial approach. A trial (Appendix 1) was outlined with the aim of: 4) identifying a method for pre-slaughter hide washing which maintained or improved carcase hygiene, without adverse effects on dark cutting or animal welfare, thus enhancing overall meat quality. This trial could not be completed during candidature due to delays in the funding contract, which were further hindered by COVID-19 and resulting restrictions on travel and on-site access to processors. Consequently, a project using historical data was identified to: 5) understand factors affecting beef carcase hygiene and identify strategies for their management to minimise risk of increased contamination during the aforementioned trial.

1.4 Thesis structure

A literature review (Chapter 2) was conducted to understand the role of pre-slaughter hide washing in the production of Australian beef. This review considered the different methodologies used across several species for pre-slaughter hide washing in Australia and internationally, their efficacy at improving visual cleanliness and microbiological contamination, as well as the effects of this practice on meat quality, and animal behaviour and welfare. This review identified a paucity of information surrounding washing practices, highlighting many gaps in our knowledge and raising more questions than it answered, thus supporting the necessity for this research.

The first research chapter (Chapter 3) examined environmental factors and animal behaviours during the pre-slaughter period and their relationship with dark cutting incidence. Several factors and animal behaviours were associated with the incidence of dark cutting. Animal behaviour was then further explored in Chapter 4 to identify factors which impact behaviour, and several environmental factors were identified. The results of these chapters further supported our initial work, suggesting a link between pre-slaughter washing, dark cutting incidence, and animal behaviour.

During data collection for the initial studies (Chapter 3 - Chapter 4), there was a noticeable behavioural change in cattle once washing commenced. Accordingly, Chapter 5 was developed to explore the behavioural response of cattle specifically to pre-slaughter hide washing treatments. This study further indicated that cattle perceive washing as a stressful event, demonstrated by a significant change in their behaviour.

With these results in hand, a large controlled trial was planned where specific washing treatments would be applied to replicate groups, allowing the effect of each treatment on meat quality, food hygiene, and animal welfare to be determined (Appendix 1). However, due to an extensive delay in contracting for funding which became further complicated by COVID-19 and the consequent restrictions on travel and site accessibility, it was not feasible to complete this study within the candidature. As the complications of COVID-19 ensued, an alternative project using historical data was identified.

Chapter 6 used data collected through the National Carcase Microbiology Monitoring Program (previously the *E. coli* and *Salmonella* Monitoring Program) to identify risk factors for beef carcase contamination. This work complemented the proposed trial (Appendix 1), ensuring factors increasing the risk of carcase contamination could be identified and managed prior to proceeding with the trial.

Finally, a general discussion (Chapter 7) collates existing knowledge from the literature (Chapter 2) with the additional knowledge gained from our experimental work (Chapter 3 - Chapter 6). A key focus of this chapter is giving consideration to proceeding with current pre-slaughter washing methods to each of the stakeholders in beef production, given our new knowledge of this practice. Directions for future work are discussed along with the implications of this thesis for industry.

Chapter 2 | Literature Review

2.1 Introduction

Food safety and hygiene play a vital role in meat production by protecting consumer health and maintaining market access. The safety and integrity of Australian beef is maintained through several standards which cover critical points in the supply chain, beginning on-farm and continuing all the way through processing and distribution. Carcase monitoring is one such standard, with *Escherichia coli* and *Salmonella spp.* being of primary concern for human health and the outbreak of human pathogenic foodborne disease. The live animal is the primary reservoir of microbial and bacterial pathogens which may be transferred to the resulting carcase during processing (McEvoy *et al.* 2003). Cattle carry these pathogens in the gastrointestinal tract, faeces, and on the hide, and their transfer to the carcase may occur directly or indirectly.

To address concerns for meat safety and public health presented by these pathogens, many preventative and curative measures are implemented in the beef supply chain, and some of these are formally required by governing authorities. The presentation of clean stock for slaughter is required under the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (Australia and New Zealand Food Regulation Ministerial Council 2007), and animals deemed not of acceptable cleanliness may be rejected for slaughter, or subject to extra processes for quality control, such as pre-slaughter hide washing. Internationally, the Code of Hygienic Practice for Meat (CODEX Alimentarius 2005) requires stock not to be loaded for slaughter when their contamination might compromise hygienic slaughter and interventions such as washing are not available. Preventative measures include the visual evaluation of live animals ante-mortem for disease and cleanliness. The hide is often acknowledged as a major source of carcase contaminants, which has made its cleanliness on the live animal an important criterion for controlling contamination (Elder *et al.* 2000; Hauge *et al.* 2012).

The objective of pre-slaughter hide washing procedures is to improve cleanliness and remove potential carcase contaminants from the animal hide. Although meat safety is of great importance and provides the rationale behind this procedure, it is only one aspect of beef production. In ensuring beef hygiene is maintained through preventative measures like pre-slaughter hide washing, it should not be to the detriment of other outcomes of processing, such as meat quality, and animal welfare, which are of increasing importance to the modern consumer. The methodologies used in this practice vary, and the effectiveness of these procedures at preventing the spread of pathogens over the hide or to the carcase is unclear. In addition, there has been little published on their effect on animal welfare and resultant meat quality. The aim of this review is to consider the role and effectiveness of pre-slaughter hide washing in Australian beef supply chains on microbial load, meat quality, and animal welfare.

2.2 Methodologies used for pre-slaughter hide washing are varied

The hide washing methodologies implemented to clean animals prior to slaughter are diverse. Those used in standard practice by industry are not formally reported, however, some information is available on the washing procedures from research trials. Given the limited available literature in this area, methods used in goats, pigs, and sheep are also considered.

The Australian Standard for the Construction of Premises Processing Animals for Human Consumption sets out that “facilities shall be provided to effectively wash or treat animals to remove contamination from the hide or skin where necessary” (Agricultural Resource Management Council of Australia and New Zealand 1995). This complements the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption, which requires that “reasonable steps are taken to present animals for inspection in a clean condition” and that “animals that are not clean are not passed for slaughter” (Australia and New Zealand Food Regulation Ministerial Council 2007). Additionally, the Operational Guidelines for the Welfare of Animals at Abattoirs and Slaughterhouses states, in relation to the washing and spraying of animals, that their use is “recommended in hot weather. In cold weather their use should be kept to a minimum” (Australian Quarantine and Inspection Service 1995). However, the National Animal Welfare Standards for Livestock Processing Establishments produced by the Australian Meat Industry Council represents the only formal document to provide guidance on how pre-slaughter hide washing procedures can and should be conducted. It simply recommends “care is taken when washing animals with high-pressure hoses to avoid sensitive areas of the animal and avoid cold stress” (Edge 2009). These guidelines are broad and unaccommodating to the many alternate washing methods available.

The ideal time for washing procedures to be conducted is not clear and as a result, many different timelines have been adopted. Washing may be conducted: as early as two days or the day before slaughter, allowing a period of overnight rest; on the day of slaughter with a few hours rest (Petersen 1983); or in some cases, left as late as 15 minutes prior to slaughter (Kannan *et al.* 2007). The later study does note that enough time should be available for the animal to dry, but highlights the importance of washing taking place within 20 minutes of slaughter in order to minimise contamination. However, it is also deemed a requirement internationally that animals should not be dripping wet at the point of slaughter. The Clean Livestock Policy in the UK classifies wet stock in the worst cleanliness categories, which requires they be rejected for slaughter (Food Standards Agency 2002).

The methods for washing are varied and include misting from above (Biss and Hathaway 1996; Walia *et al.* 2017), power-hosing (Byrne *et al.* 2000; Kannan *et al.* 2007), swim washing (Petersen 1983; Biss and Hathaway 1996), belly washing (Biss and Hathaway 1996), commercial cattle washing systems which apply water from above and beneath the animal (Mies *et al.* 2004), or a combination of these treatments. Misting has been described in pigs and sheep where water is delivered through spray nozzles or pipes located above the holding pen, or in a specialised washing pen (Biss and Hathaway 1996; Walia *et al.* 2017). Power-hosing utilises a hose with a single nozzle hole held approximately 30cm from the animal and is operated by a person to clean the entire body surface of the animal (Byrne *et al.* 2000; Kannan *et al.* 2007; Preston *et al.* 2016). This method has been described in cattle and goats. Swim washing procedures have only been reported for use with sheep. Baths for swimming range in size from 113cm wide x 1560cm long x 70cm deep (Petersen 1983) to 1.5m wide x 20m long x 1.5m deep (Biss and Hathaway 1996) and sheep enter at one end, swim the length of the bath, and exit the opposite end. Belly washing is conducted in pens specially fitted with spray nozzles in the floor, aimed at the belly of the animal, and has been used in cattle (Preston *et al.* 2016) and sheep (Biss and Hathaway 1996). One study described an automated, commercial cattle washing system, which delivered water in a cycle from beneath the animals for 30 seconds, and then above the animals for 30 seconds, and more than one cycle may be used (Mies *et al.* 2004). The use of some of these practices have also been described in combination.

These washing procedures are generally conducted on a group of animals and the extent of restraint used during washing is typically limited to the size of the pen, with animals being able to move around freely within. The exception to this is a procedure described in goats that were power-hosed individually whilst restrained in a single file race (Kannan *et al.* 2007). The duration of washing varies between methods, the shortest being swim washing which lasts for as little as 30-40 seconds (Petersen 1983). A period of one to five minutes is most commonly described, and includes studies of power-hosing (Byrne *et al.* 2000; Kannan *et al.* 2007), the commercial cattle wash system (Mies *et al.* 2004), and belly washing (Biss and Hathaway 1996). The longest treatment described was misting from above in pigs which lasted 30 minutes (Walia *et al.* 2017). The duration of some formally studied treatments were not reported.

Potable water alone is most common for washing, however additives can also be used and include Virkon® S (Walia *et al.* 2017), L-lactic acid, and chlorine (Mies *et al.* 2004). Water is delivered at 10-18°C (Byrne *et al.* 2000; Kannan *et al.* 2007) at rates of 12-15 litres per minute from a power-hose (Byrne *et al.* 2000; Kannan *et al.* 2007) to 780-1020 litres per minute in a belly wash (Biss and Hathaway 1996), up to 1325 litres per minute in the commercial cattle washing system (Mies *et al.* 2004). Swim washing systems use from 34 to 60 m^3 per hour with fresh water entering the system from the point at which the animals exit (Biss and Hathaway 1996). Delivery of water has been recorded at 1034 kPa from pressure-hoses and in the commercial cattle washing system (Byrne *et al.* 2000; Mies *et al.* 2004).

It is evident there is considerable variation in the methods used to clean slaughter stock and this may contribute to inconsistencies in the results of washing trials. The methodologies themselves differ in their set up and function, as well as the point at which they occur prior to slaughter, their duration, and the nature of water delivery, including the use of additives. The methods used in commercial facilities are not reported, and whilst these studies provide an indication of such methods, the extent and normal application of these under non-scientific protocols is not known. Knowledge of pre-slaughter hide washing procedures as they are routinely conducted in industry would be beneficial, allowing the success of such treatments to be clearly understood and to provide a more directed guide for further studies, and ultimately the necessity of this practice.

2.3 Pre-slaughter washing improves visible cleanliness of the animal hide and carcase, at the expense of microbial contamination

One of the factors underpinning the process of washing animals prior to slaughter is that their cleanliness is visually improved, thus lowering the likelihood of potential contaminants being present, or spreading from the hide to the carcase. There is evidence of improvements in visual cleanliness of both the hide and subsequent carcase following pre-slaughter washing, but literature showing this lacks a formal definition or scale of cleanliness (Biss and Hathaway 1996; Bell 1997a; Byrne *et al.* 2000; Kannan *et al.* 2007; Walia *et al.* 2017).

Evaluating the effect of pre-slaughter hide washing on visible animal cleanliness would ideally include an evaluation of the effectiveness of such procedures. This would be possible by first defining states of cleanliness categorically, and then recording the cleanliness of the animal before and after completion of washing treatments. One of the more thorough references defining and categorising cleanliness is provided by the Food Standards Agency in the United

Kingdom through the Clean Livestock Policy (Food Standards Agency 2002). This policy describes cleanliness categorically on a one to five scale, providing both written and visual reference for cattle and sheep fitting each category. In general, animals tend to be described as unclean when attachments such as faeces, mud, or bedding (tags) are present on the coat. The inclusion of a cleanliness definition and scoring system would help facilitate the comparison between reports and treatment methods.

The literature evaluating the improvement in visible animal cleanliness from washing, whilst small, shows a positive effect (Biss and Hathaway 1996; Bell 1997a; Byrne *et al.* 2000; Kannan *et al.* 2007; Walia *et al.* 2017). However, a measure of the improvement resulting from the different methods is not provided, other than to say animal cleanliness improved. For example, Walia *et al.* (2017) reported that misting from above for 30 minutes with water 15–17°C at 200 kPa resulted in pigs that looked visibly clean. Both Byrne *et al*. (2000) and Kannan *et al.* (2007) were able to produce visibly clean cattle and goats respectively, following washing for one minute with a power hose. In lambs, Biss and Hathaway (1996) reported an improvement in visible contamination of sheep following misting from above, swim washing and belly washing. A study by Bell (1997b) reported that washing cattle prior to slaughter removed large faecal attachments, however specific details of the washing method used was not provided. The effect of washing the animal on visible carcase contamination has also been reported, where carcases from washed sheep had less visible contamination than those from unwashed sheep (Biss and Hathaway 1996). Macro contaminants such as faeces, dirt, sand, and wool were also more evident on carcases derived from unwashed animals (Biss and Hathaway 1996).

The available literature suggest that pre-slaughter hide washing methods are an effective way to improve the visible cleanliness of slaughter stock. This body of literature would be greatly aided by defining visible cleanliness and the categories it may take, such as is provided by the Clean Livestock Policy, and recording the cleanliness of animals before and after washing treatments, to determine treatment effectiveness.

2.3.1 Visible cleanliness and microbial contamination are related

The necessity to couple measuring improvement in visible cleanliness with the level of microbiological contamination of both the hide and carcase is evident, given literature suggests improvements in visible cleanliness do not correspond to improvements in microbiological cleanliness (Byrne *et al.* 2000; Kannan *et al.* 2007), and may in fact be to its detriment (Biss and Hathaway 1996).

A relationship between visible cleanliness of the animal and microbiological cleanliness has been reported in several studies. Byrne *et al.* (2000) found that power-hosing cattle for one or three minutes improved visual cleanliness and reduced faecal contamination on hides. However, there was no difference in the *E. coli* counts obtained from carcases from unwashed, one minute or three minute washed animals. Kannan *et al.* (2007) found that skin aerobic plate counts were significantly less ($P<0.05$) in goats that had been power-hosed for one minute than those receiving no treatment, although this was not true for *E. coli* counts on the hide where no difference was found. Interestingly however, this study also found that washing had no effect on subsequent carcase contamination, measured as aerobic plate counts and *E. coli* counts, even though visible cleanliness of the animal improved. These findings are further supported by Biss and Hathaway (1996), who reported the effect of washing sheep prior to slaughter on the visible cleanliness of animals and microbiological contamination of carcases. An improvement in the visible contamination of lambs was associated with an increase in the aerobic plate count of carcases. Carcases from washed sheep, regardless of wool length, had higher aerobic plate counts and *E. coli* counts than those from unwashed sheep (Biss and Hathaway 1996). Together, these studies show that whilst washing results in an animal that is visually acceptable in terms of cleanliness following pre-slaughter hide washing, the effect on the subsequent carcase cleanliness is, at best, negligible if not worsened. Thus, the importance of visual cleanliness requires further investigation to determine its appropriateness as a measure of actual cleanliness, and its continued role in maintaining beef hygiene.

2.4 Pre-slaughter washing does not control microbiological contamination on the hide or carcase

Microbiological contamination is a major concern to the safe production of beef given the potential for serious consequences to human health. As such, prevention schemes such as the National Carcase Microbiological Monitoring Program (NCMMP; formerly the *E. coli* and *Salmonella* Monitoring Program) have been introduced to monitor carcase hygiene in Australia.

When considering the effectiveness of pre-slaughter hide washing at controlling or reducing microbiological contamination, the literature findings highlight the importance of distinguishing whether this is being evaluated on the animal hide or resulting carcase. It would appear logical that the effectiveness of such procedures should be evaluated on the hide itself, given this is where they are applied. However, hide removal is a major point of carcase contamination in the post-slaughter environment. Contaminants spread more easily from wet animals (Bell 1997b), which are the product of pre-slaughter hide washing, and differences in contamination of the hide and carcase following washing could be possible. Thus, in examining the success of washing methods in controlling microbiological contamination, their effectiveness on both the hide and carcase are considered. Table 2.1 summarises the literature reporting the effects of pre-slaughter hide washing practices on microbiological contamination of applied to cattle, sheep, goats, and pigs.

Table 2.1 Summary of literature findings reporting the effect of pre-slaughter hide washing treatments on contamination in cattle, sheep, pigs and goats.

Contaminant	Washing treatment	Pre-washing	Post-washing	Result and comments	Reference
		Cattle			
Escherichia coli	Potable water wash (10-18°C) using a powerhose delivering 15l of water per minute at 150psi. Washed for 1 minute with nozzle held 30cm from the animal.	Sample site: Hide: $50cm^2$ area swabbed using MRD moistened cotton wool swabs. Sample time: Prior to slaughter, 24 hours after *E. coli* inoculated faeces applied to hide. Contamination: 1.9×10^2 CFU/cm^2	Sample site: Hide: $50cm^2$ area swabbed using MRD moistened cotton wool swabs. Sample time: Post-washing. Contamination: 1.0×10^2 CFU/cm^2	Not significantly different to the unwashed control group. Unwashed control and treatment groups were not significantly different.	(Byrne *et al.* 2000)
			Sample site: Carcase: measured area of forequarter and hindquarter swabbed using MRD cellulose sponge. Sample time: Not defined. Contamination: 1.0×10^2 CFU/cm^2	Not significantly different to the unwashed control group. Unwashed control and treatment groups were not significantly different.	(Byrne *et al.* 2000)
	Potable water wash (10-18°C) using a powerhose delivering 15l of water per minute at 150psi. Washed for 3 minutes with nozzle held 30cm from the animal.	Sample site: Hide: $50cm^2$ area swabbed using MRD moistened cotton wool swabs. Sample time: Prior to slaughter, 24 hours after *E. coli* inoculated faeces applied to hide. Contamination: 1.9×10^2 CFU/cm^2	Sample site: Carcase: measured area of forequarter and hindquarter swabbed using MRD cellulose sponge. Sample time: Not defined. Contamination: 2.3 CFU/cm^2	Non-significant decrease. Unwashed control and treatment groups were not significantly different.	(Byrne *et al.* 2000)
Salmonella	Automatic commercial cattle wash applying 1,325l of water over a 30 second period beneath cattle followed by a 30 second period from above.	Sample site: Hide: $100cm^2$ area sampled using a premoistened sponge on the left hand side of the animal in specified areas. Sample time: Prior to washing. Contamination: 57.8%	Sample site: Hide: $100cm^2$ area sampled using a premoistened sponge on the right hand side of the animal in specified areas. Sample time: 15 minutes posts washing. Contamination: 72.2%	Increase. Significance not determined. Washing treatments including L-lactic acid, acetic acid, and chlorine were not significantly different from water only ($p<0.05$).	(Mies *et al.* 2004)
	Automatic commercial cattle wash applying two washes of 1,325l of water over a 30 second period beneath cattle followed by a 30 second period from above.	Sample site: Hide: $100cm^2$ area sampled using a premoistened sponge on the left hand side of the animal in specified areas. Sample time: Prior to washing. Contamination: 35.6%	Sample site: Hide: $100cm^2$ area sampled using a premoistened sponge on the right hand side of the animal in specified areas. Sample time: 15 minutes posts washing. Contamination: 40.0%	Increase. Significance not determined. Washing treatments including L-lactic acid, acetic acid, and chlorine were not significantly different from water only ($p<0.05$).	(Mies *et al.* 2004)

Aerobic plate counts, coliforms, *Escherichia coli*	Automatic commercial cattle wash applying 1,325l of water over a 30 second period beneath cattle followed by a 30 second period from above.	<u>Sample site:</u> Hide: 100cm^2 area sampled using a premoistened sponge on the left hand side of the animal in specified areas. <u>Sample time:</u> Prior to washing. <u>Contamination:</u> 0.1 $\log_{10}$ CFU/cm^2	<u>Sample site:</u> Hide: 100cm^2 area sampled using a premoistened sponge on the right hand side of the animal in specified areas. <u>Sample time:</u> 15 minutes posts washing. <u>Contamination:</u> 0.8 $\log_{10}$ CFU/cm^2	Increase. Significance not determined. Washing treatments including L-lactic acid, acetic acid, and chlorine were not significantly different from water only ($p<0.05$).	(Mies *et al.* 2004)
	Automatic commercial cattle wash applying two washes of 1,325l of water over a 30 second period beneath cattle followed by a 30 second period from above.	<u>Sample site:</u> Hide: 100cm^2 area sampled using a premoistened sponge on the left hand side of the animal in specified areas. <u>Sample time:</u> Prior to washing. <u>Contamination:</u> 0.1 $\log_{10}$ CFU/cm^2	<u>Sample site:</u> Hide: 100cm^2 area sampled using a premoistened sponge on the right hand side of the animal in specified area. <u>Sample time:</u> 15 minutes posts washing. <u>Contamination:</u> 0.8 $\log_{10}$ CFU/cm^2	Increase. Significance not determined. Washing treatments including L-lactic acid, acetic acid, and chlorine were not significantly different from water only ($p<0.05$).	(Mies *et al.* 2004)
			Sheep		
Aerobic plate count	A swim wash using 34-60m^3 of potable water per hour, made of a concrete trough approximately 20m long, 1.5m deep, 1.5m wide. Some lambs washed for 2-5 minutes prior to entering the swim wash in pens containing 156-204 nozzles delivering 780 and 1020l of water per minute.	Not recorded	<u>Sample site:</u> Carcase: ten samples excised from each carcase at specified locations were pooled in a Seward 400 stomacher bag for processing. <u>Sample time:</u> Immediately after pelting and before trimming. <u>Contamination:</u> 4.39 – 5.46 $\log_{10}$/cm^2	Significant increase ($p<0.001$) compared to the unwashed control which ranged from 3.74 – 4.67 $\log_{10}$/cm^2	(Biss and Hathaway 1996)
Escherichia coli	A swim wash using 34-60m^3 of potable water per hour, made of a concrete trough approximately 20m long, 1.5m deep, 1.5m wide. Some lambs washed for 2-5 minutes prior to entering the swim wash in pens containing 156-204 nozzles delivering 780 and 1020l of water per minute.	Not recorded	<u>Sample site:</u> Carcase: ten samples excised from each carcase at specified locations were pooled in a Seward 400 stomacher bag for processing. <u>Sample time:</u> Immediately after pelting and before trimming. <u>Contamination:</u> 0.82 – 2.34 $\log_{10}$/cm^2	Significant increase ($p<0.01$) compared to the unwashed control which ranged from 0.15 – 1.23 $\log_{10}$/cm^2	(Biss and Hathaway 1996)
			Pigs		

Salmonella	Cold water (15-17°C) misting from an overhead pipe supplying 1.4L of water per minute. Washed for 30 minutes in lairage pens prior to slaughter.	*Sample site:* Hide: head to tail using a 10cm maximum recovery dilutant swab (coverage area = 10cm swab x 160cm estimated body length). *Sample time:* On entry to lairage. *Contamination:* Prevalence = 72.1%	*Sample site:* Hide: head to tail using a 10cm maximum recovery dilutant swab (coverage area = 10cm swab x 160cm estimated body length). *Sample time:* Pre-stunning. *Contamination:* Prevalence = 94.3%	Significant increase ($p<0.05$). No change in prevalence was detected in the unwashed control group.	(Walia *et al.* 2017)
Enterobacteriaceae	Cold water (15-17°C) misting from an overhead pipe supplying 1.4L of water per minute. Washed for 30 minutes in lairage pens prior to slaughter.	*Sample site:* Hide: head to tail using a 10cm maximum recovery dilutant swab. Sample area = 10cm swab x 160cm estimated body length). *Sample time:* On entry to lairage. *Contamination:* Mean from 15 animals = 3.31 log_{10} CFU/cm^2	*Sample site:* Hide: head to tail using a 10cm maximum recovery dilutant swab (coverage area = 10cm swab x 160cm estimated body length). *Sample time:* Pre-stunning. *Contamination:* Mean from 15 animals = 5.62 log_{10} CFU/cm^2	Significant increase ($p<0.05$). No change in prevalence was detected in the unwashed control group.	(Walia *et al.* 2017)
			Goats		
Escherichia coli	Potable tap water (15-18°C) applied from a spray nozzle fitted to a hose delivering 12L of water per minute. Washed individually for 1 minute evenly over the body, excluding the head.	*Sample site:* Hide: right hind leg using a sponge hydrated with buffered peptone water. Sample area = 5cm x 5cm template. *Sample time:* Prior to washing. *Contamination:* 2.5 log_{10} CFU/cm^2	*Sample site:* Carcase: left hind leg using a sponge hydrated with buffered peptone water. Sample area = 5cm x 5cm template. *Sample time:* Immediately after skinning and pre-evisceration. *Contamination:* 2.1 log_{10} CFU/cm^2	Non-significant reduction ($p = 0.57$). Counts for both washing and unwashed control groups were reduced to the same level.	(Kannan *et al.* 2007)
Aerobic plate count	Potable tap water (15-18°C) applied from a spray nozzle fitted to a hose delivering 12L of water per minute. Washed individually for 1 minute evenly over the body, excluding the head.	*Sample site:* Hide: right hind leg using a sponge hydrated with buffered peptone water. Sample area = 5cm x 5cm template. *Sample time:* Prior to washing. *Contamination:* 4.6 log_{10} CFU/cm^2	*Sample site:* Carcase: left hind leg using a sponge hydrated with buffered peptone water. Sample area = 5cm x 5cm template. *Sample time:* Immediately after skinning and pre-evisceration. *Contamination:* 4.0 log_{10} CFU/cm^2	Non-significant reduction ($p = 0.87$). Counts for both washing and unwashed control groups were reduced to the same level.	(Kannan *et al.* 2007)

2.4.1 The hide

The hide has been identified as a major source of carcase contaminants (Elder *et al.* 2000). Examining the effectiveness of pre-slaughter washing methods at removing microorganisms from the hide is thus likely to provide an indication of the potential for subsequent contamination of the carcase, given their positive correlation (Elder *et al.* 2000).

Pre-slaughter washing treatments can facilitate contamination of the hide. Studies in cattle and pigs reported increases in Enterobacteriaceae, including *Salmonella* and *E. coli*, aerobic plate counts, and coliforms following washing with water alone, lactic acid, and chlorine solutions, despite animals appearing visually clean (Mies *et al.* 2004; Walia *et al.* 2017). In comparison, when Walia *et al.* (2017) applied no treatment to pigs, *Salmonella* and other Enterobacteriaceae counts on the animal hide did not change significantly, suggesting washing with water actually aided the dispersal and distribution of contaminants. Furthermore, the contamination rates of wet animals are similar to those recorded for unwashed animals that are dirty but dry (Byrne *et al.* 2000).

Other studies have reported a reduction in *E. coli* and aerobic plate counts following hide washing treatments. When goats and cattle were power-hosed for one and three minutes, respectively, their corresponding hide aerobic plate counts and *E. coli* counts significantly decreased (Byrne *et al.* 2000; Kannan *et al.* 2007). Whilst a decrease in *E. coli* was also observed on the hides of cattle power-hosed for three minutes, the number transferred to the subsequent carcase was not reduced (Byrne *et al.* 2000). This was also observed in goats, where *E. coli* counts did not change significantly following the one minute washing treatment (Kannan *et al.* 2007). Cattle receiving a shorter power-hose of just one minute also had similar levels of contamination to unwashed animals that were dirty but dry (Byrne *et al.* 2000).

The results of these limited studies indicate that of the methodologies tested, the most effective washing treatments have little to no effect on improving the microbiological contamination of the hide, and some in fact worsen it. These studies suggest power-hosing the hide is less detrimental to contamination than misting and soaking treatments. Hide washing appears to facilitate the spread of contaminants from heavily affected areas of the hide, where they would otherwise be fixed, to all locations on the hide surface (Mies *et al.* 2004). The hides of washed and unwashed animals are considered microbiologically equivalent prior to treatment (Kannan *et al.* 2007), therefore hides returning a positive result post treatment are likely to have been contaminated prior to washing, but only in a smaller, non-detected area. This suggests that washing favours the spread of contamination, increasing the area over which contaminants are located and thus increasing the likelihood for detection, rather than necessarily the introduction of new contaminants. These studies considered selected pre-slaughter hide washing treatments, and there is need for further work examining the effectiveness of all washing methodologies in reducing microbiological contamination on the hide.

2.4.2 The carcase

Whilst pre-slaughter washing treatments are applied to the hide, their effect on the subsequent carcase is of primary concern. Washing inherently wets the hide, and if animals remain wet at the time of slaughter, the transfer of contaminants during the hide removal process may be increased (Bell 1997b). One study found cattle with hides soiled with faecal matter that were washed prior to slaughter yielded carcase with more contamination than those derived from clean, unwashed cattle (Bell 1997b). When compared to dry, unwashed animals, carcases derived from washed cattle were overall similarly contaminated when power-hosed for one or three minutes (Byrne *et al.* 2000). This was further supported by Kannan *et al.* (2007)

who power-hosed goats for one minute, and found no significant difference in carcase aerobic plate counts or *E. coli* counts when compared to unwashed animals. These studies indicate the best outcome of pre-slaughter washing on carcase contamination is no effect, however, contamination may increase following washing treatment.

Studies in sheep have also shown carcases derived from washed animals tended to have greater bacterial contamination than those from unwashed animals (Biss and Hathaway 1995). A later study by Biss and Hathaway (1996) further supported this, finding total aerobic bacteria and *E. coli* counts to be greater on carcases from lambs that had been washed prior to slaughter, regardless of wool length. The effect of washing on microbial carcase contamination is also related to wool condition, with woolly animals returning greater increases in microbial carcase contaminants following washing than those which had been shorn (Biss and Hathaway 1996). In contrast, another study of sheep, which defined hide cleanliness categorically, found carcase contamination with aerobes and Enterobacteriaceae increased as fleece cleanliness decreased (Hadley *et al.* 1997).

Whilst the literature examining the effectiveness of pre-slaughter hide washing on carcase contamination is relatively small, it is generally consistent in suggesting that such procedures are not effective in meeting their intended outcome. Gill (2004) also concluded that washing the animal was an ineffective meat safety measure from a microbiological perspective, finding the best result of washing treatments on the microbiological status of the carcase was that they had no effect, but at worst they may contribute to increasing contamination. There is much scope for further work in this area, and it is imperative that the effectiveness of pre-slaughter hide washing procedures are determined to ensure they are fit for purpose and not contributing to the contamination of carcases, particularly given their potential for additional effects on meat quality and animal behaviour.

2.5 Pre-slaughter hide washing negatively affects meat quality

The effect of pre-slaughter hide washing on meat quality deserves consideration to ensure that beef produced is both safe to consume and meets consumer expectations for eating quality. Meat quality defects common in beef production include dark cutting, ecchymosis and bruising. These defects can result in a product with poor eating quality attributes, a lack of visual appeal, and reduction in saleable lean meat, respectively.

Petersen (1983) and Preston *et al.* (2016) both showed the number of washes received was related to elevated pH of lamb and beef, respectively. The initial study by (Petersen 1983) recorded the number of times sheep were washed by means of swimming through a 1560cm bath, and found a linear relationship between washing and ultimate pH (pH_u). The pH_u increased with the number of washes a group received, as did the range in pH_u values, and this was irrespective of a resting period. Preston *et al.* (2016) reported a similar relationship after observing cattle washed using a combination of belly washing and power-hosing. In this study, the number of belly washes was positively related to pH and dark cutting incidence, with each wash received increasing dark cutting incidence within the group by as much as five percent. Additionally, Walker *et al.* (1999) found a significant effect of washing on muscle glucose and lactate concentration when compared to unwashed controls. Cattle in this study were first washed 30 minutes prior to slaughter using in-floor sprays for 40 seconds, followed by high-pressure hosing which lasted up to 5 minutes and was conducted approximately 10 minutes prior to slaughter (Walker *et al.* 1999). In summer, glucose concentration was 0.63mg/g less in the *semimembranosus* of washed cattle compared to unwashed controls (Walker *et al.* 1999). In winter, lactate concentration was 0.61mg/g and 0.70mg/g less in the *M. semimembranosus* and *M. semitendinosus*, respectively, of washed cattle (Walker *et al.* 1999). To the best of our knowledge, no other studies have reported the effect of pre-slaughter hide washing on meat quality.

The effect of washing on other carcase quality attributes, such as bruising and ecchymosis, requires investigation. Animal movement in wet pens and changes in behaviour from washing, such as increased mountings or avoidance of water sprays, may result in slipping, falling, or physiological changes from stress which could lead to bruising or ecchymosis. Factors affecting meat quality such as these are of great importance, particularly due to their potential detrimental effect on carcase value. While just two reports, there is evidence that washing has negative effects on ultimate pH and meat colour (Petersen 1983; Preston *et al.* 2016). It follows that the effectiveness of pre-slaughter hide washing at reducing carcase contamination needs evaluation to justify its necessity and the appropriateness of current methods.

2.6 Pre-slaughter washing changes animal behaviour, suggesting welfare is adversely affected

Whilst the effect of washing the live animal on behaviour and welfare has not been thoroughly investigated, there is some evidence of a change in behaviour in response to washing procedures, and these behaviours can be indicative of stress.

Greenwood *et al.* (2000) investigated biological indicators of stress (in the blood: plasma cortisol, plasma lactate, and creatine kinase; in the muscle: glycogen and lactate), animal vocalisations and observations of agitation and movement ease. This study observed lot fed steers washed by a rotating drum for 60 seconds, a shearing treatment, or group washing involving soaking and hosing in pens for four hours. Blood samples were collected 30 minutes post-treatment; cattle were then kept in clean pens for one week before slaughter, and muscle sampling occurred 30 minutes post-slaughter. The effect of four-hour washing on animal vocalisations and behavioural measures was not reported, however, these measures were unaltered in the shorter duration rotating drum and shearing treatments when compared to the control. Greenwood *et al.* (2000) also noted the need to further investigate the extent to which washing stressed animals.

Preston *et al.* (2016) observed pasture and grain finished cattle held in lairage and recorded their behaviour throughout this time, including while being washed. The number of times cattle were observed mounting was strongly positively correlated with the number of times a group was washed with the high-pressure hose. This observation was not correlated to the number of times mountings were recorded during other points of observation, suggesting the behaviour was more likely a fear response to environment rather than group characteristics. The greatest number of mountings were recorded while cattle were being washed with the high-pressure hose, and cattle were also observed attempting to avoid the hose.

Kannan *et al.* (2007) recorded plasma cortisol, glucose, and non-esterified fatty acid concentration, and noted change in animal behaviour following the washing of 20 meat goats which were each spray washed for one minute with potable water prior to slaughter. There was a higher concentration of plasma cortisol, glucose, and non-esterified fatty acids following washing from basal levels; however, this increase was not significantly different between the washed and unwashed control group, which may have simply been due to experimental group size. This study did not formally record animal behaviour, but the authors noted that "during the spray washing treatment, the animals tended to move during the initial seconds in an effort to avoid the water spray" (Kannan *et al.* 2007).

Water spraying has also been reported as a stressor in dairy cattle, where animals are sprayed to control heat stress. While the purpose of these washing treatments is to cool the animal during high heat load rather than improve their cleanliness, behavioural responses indicating stress have been recorded. Cattle spent more time with their head down when exposed to sprinklers used for cooling, indicating aversion (Kendall *et al.* 2007; Chen *et al.* 2016a, 2016b). It has also been shown that cattle prefer areas without sprinklers (Schütz *et al.* 2010), and will visit areas with sprinklers less frequently (Chen *et al.* 2016a). Similar to the above-mentioned studies, spray treatment did not result in immediate physiological changes (insulin and metabolites). However, when followed postpartum, cattle treated with sprinklers had lower plasma glucose and insulin concentration, and increased non-esterified fatty acid (Tao *et al.* 2012). These observations of aversive behaviours in response to washing support the hypothesis that washing is a stressful process, highlighting the need for continued monitoring of behaviour in such studies.

These preliminary studies substantiate the need for further research to conclusively determine the effect of commercial pre-slaughter washing treatments on animal behaviour and welfare. Whilst Greenwood *et al.* (2000) suggested prolonged washing was a less stressful treatment based on blood indicators, this study was conducted in lot fed cattle which have high glycogen stores compared to pasture finished cattle (Immonen *et al.* 2000), and which more regularly interact with humans. This may provide an explanation for why no increase was reported in biological indicators due to the washing treatment. However, a decrease in feed intake has been observed following washing treatments applied at a feedlot, suggesting treatments may still be perceived as a stressor (Wockner and Jewell 2019). In their study of meat goats, Kannan *et al.* (2007) reported an non-significant increase in plasma cortisol in washed compared to unwashed animals, and a change in behaviour similar to that reported by Preston *et al.* (2016). Avoidance of stimuli, like the behaviour described in these studies, is an indicator of fear in animals (Romeyer and Bouissou 1992) suggesting the washing treatments were a stressful event.

It is quite possible that the studies reported herein may not provide an accurate representation of industry washing practices. Commercially, cattle are power-hosed for a duration much longer than one minute, and washing typically occurs throughout the day of slaughter (Preston 2015), rather than a week or only 15 minutes prior as in the studies by Greenwood *et al.* (2000) and Kannan *et al.* (2007), respectively. Further studies are needed to determine the effect washing practices, their methodology, duration, frequency, and timing, have on the behaviour and welfare of slaughter stock. Recording of animal behaviour has proved a useful, non-invasive method to assess the welfare of animals and can be easily applied to further studies. The slaughter of stock following washing, if completed in a suitable timeframe, may also present the opportunity to sample physiological measures of stress, such as blood and muscle indicators, without causing stress to the live animal.

2.7 The continued use of pre-slaughter hide washing is questionable

The continued use of pre-slaughter hide washing as a measure of preventing or limiting microbiological contamination of the carcase requires careful consideration. The relevant standards and regulations both within Australia and our international trade markets necessitate pre-slaughter hide washing, or an equivalent process, to ensure requirements for the cleanliness of slaughter stock are met.

The animal itself is host to, and the primary source of, human enteric pathogens of concern in beef production, particularly *E. coli* and *Salmonella*. The hide is the primary source of these contaminants, partly due to faecal loading, and the most likely point for contamination of the carcase is during the hide removal process. If the animal is wet, there is even greater potential for contamination. Whilst other sections of carcase processing play a role in, and may contribute to contamination, it is more likely this results from cross-contamination rather than the introduction of new pathogens. Pre-slaughter washing may favour the spread of these contaminants over the hide and increase the probability of their transfer and detection on the carcase. Thus, the importance of the animal hide in the prevention of contamination, and the need to manage the animal accordingly can be appreciated. In regard to this, pre-slaughter hide washing is a popular method within Australia to ensure the cleanliness of stock reaches an acceptable standard on visual evaluation.

The methodologies by which pre-slaughter hide washing can be conducted are varied and facilitate an improvement in the visible cleanliness of animals. However, it is apparent that the effect of these procedures on the microbiological cleanliness of both the hide and resulting carcase is negligible and may in fact worsen contamination. The introduction of water to the hide likely dislodges contaminants already present from their otherwise fixed position, enabling their spread over a wider area of the hide, and increasing their potential for cross contamination on contact. Whilst the concentration of contaminants may be lessened on dispersal more widely over the hide, the nature of the pathogens of concern to human health is such that only a small number are required to cause severe disease. Whilst food safety and meat hygiene are of significant importance, they represent only one aspect of beef production. Pre-slaughter hide washing techniques have been identified as detrimental to meat quality, particularly dark cutting beef, and are in conjunction with studies finding these procedures to be stressful to the animal.

Overall, there is a paucity of information available in the literature in relation to pre-slaughter hide washing across many species. The effectiveness and suitability of this practice is at best negligible, and its continued use is questionable. The author acknowledges such procedures are unlikely to be ceased given present national and international market regulations, however a review of pre-slaughter hide washing practices are necessary to optimise their effectiveness at maintaining, or at least not worsening, microbiological contamination of the carcase such that they are not also detrimental to meat quality or behaviour and welfare of the animal. From the available literature with consideration for beef production from a broad perspective, one can only be left asking: ‘why are we washing cattle?’

Chapter 3 | Pre-slaughter factors affecting dark cutting in pasture finished beef cattle

3.1 Introduction

Dark cutting, or dark, firm, dry beef, is a significant problem affecting meat quality. Dark cutting beef is typically dark in colour (Hughes *et al.* 2020), has variable tenderness (Purchas and Aungsupakorn 1993), a high water holding capacity (Lawrie and Ledward 2014), and is therefore prone to bacterial spoilage, which reduces shelf life (Borch *et al.* 1996). Consumers negatively discriminate against dark coloured meat (Troy and Kerry 2010), which is the most important attribute influencing likelihood to purchase (Carpenter *et al.* 2001). These inferior quality attributes and unacceptability with consumers have resulted in dark cutters being ineligible to receive a Meat Standards Australia (MSA) grade. As a result, the price paid to producers for dark cutting carcases is discounted by, on average, \$0.59/kg Hot Standard Carcase Weight (HSCW) (Jose *et al.* 2015). This problem represents a great burden to the beef industry, making a reduction in dark cutting of significant importance.

In Australia, dark cutting carcases are defined by a pH greater than 5.71 (Thompson 2002) and, until July 2017 (Meat and Livestock Australia 2017), a meat colour darker than AUS-MEAT colour 3 (Watson *et al.* 2008). Dark cutting is the result of insufficient levels of muscle glycogen at slaughter, the main cause of which is stress (Tarrant 1989). The amount of muscle glycogen available at slaughter is a function of stored glycogen, resulting from nutrition, and any glycogen metabolised during the pre-slaughter period in response to stressors, especially once the animal is taken off feed (Gardner *et al.* 2014). In cattle, resting muscle glycogen concentration is approximately 80µmol/g, and dark cutting occurs when this level falls below 57µmol/g at slaughter (Tarrant 1989). Glycogen breakdown can occur at a rate of up to 11 µmol/g/hour, depending on the activating stressor (Tarrant 1989). However, in fasted cattle, muscle glycogen repletion occurs at a rate of just 0.06µmol/g/hour (Tarrant 1989). Thus, any stressors initiating glycogen breakdown in the pre-slaughter period will have irreversible effects on meat quality.

The effect of chronic stress on meat quality is well documented, however comparatively little is known about acute stress and dark cutting. Abattoir lairages contain many factors which may activate the stress response, decreasing muscle glycogen availability at slaughter (Terlouw 2005). Stressors can be any stimuli, either real or perceived, which activate the hypothalamic-pituitary-adrenal (HPA) axis to initiate the stress response (Veissier and Boissy 2007). Following activation of the HPA axis, glucocorticosteroids including cortisol are released, altering energy metabolism (Ferguson and Gerrard 2014). Corticotropin releasing hormone and adrenocorticotrophic hormone levels increase, increasing glucagon secretion and stimulating glucogenolysis (Sapolsky *et al.* 2000).

In addition to physiological responses, stressors can also result in behavioural changes. Animal behaviour is a cheap, non-invasive tool available to investigate stress given its associations with physiological parameters (Hemsworth *et al.* 2011). The observation of behaviour reduces the need for invasive techniques like blood sampling, which impose additional stress on the animal (Zavy *et al.* 1992; María *et al.* 2004). Consequently, the performance of certain behaviours can be used to assess the state of an animals welfare, and indicate when this is threatened.

The pre-slaughter period is a critical phase for meat quality; there is no opportunity to replenish any glycogen stores that are metabolised during this period. This study investigated the relationship between pre-slaughter factors in lairage, including environmental factors and behaviour, and dark cutting incidence. We hypothesised that stressful stimuli which cattle are exposed to during lairage would be associated with an increase in dark cutting incidence.

3.2 Materials and methods

Data for this observational cohort study were collected over 21 kill days from a large Southern Australian abattoir during June 2015 to November 2017. Cattle from commercial mobs were selected randomly from those scheduled to be processed. The only requirement for inclusion in this study was that cattle would be presented for Meat Standards Australia grading and be certified pasture finished. This project was approved by The University of Adelaide Animal Ethics Committee (Approval number S-2016-096).

3.2.1 Animal management

Cattle (n=3,054) arrived at the facilities the day prior or morning of slaughter where they were unloaded and housed in lairage pens. Each lairage pen could accommodate 20 animals, with the exception of two larger pens which each held 50 animals. Thus, most often larger consignments (>20 head) needed to be separated into smaller groups. No other mixing of mobs occurred. Once mobs were separated into lairage pens, they remained in these groups for processing, and these groups formed the unit of replication for this study (wash group). On the morning of slaughter cattle were washed to remove contaminants (mud, faeces, and bedding) from the hide. Slaughter was then conducted by captive bolt gun stun followed by exsanguination.

3.2.2 Unloading

Animals were observed and behaviour recorded during unloading. During 2015 cattle were unloaded using a single ramp of adjustable height which only accommodated unloading from the rear of the truck. The ramp was wide enough to comfortably allow animals to unload in single file and had concrete flooring with square tubing slats. A new ramp was installed in early 2016 following an upgrade, after which the older ramp was no longer used. The new ramp allowed rear and side unloading, and was wide enough that animals could pass each other on the ramp, which had grooved concrete flooring. The time taken to unload was recorded from the point the truck gate was opened and animals could leave the crate, until the last animal had descended the ramp. The type of truck (semi, b-double, other) was also recorded, including the number of decks, and number of cattle on the truck. The behaviours observed were those described by María *et al.* (2004) (Table 3.1), and these were recorded on both ramps from the time animals left the crate at the top of the ramp, until the last animal had reached the bottom of the ramp. Behaviours in the unloading pens at the bottom of the ramp were not recorded. Cattle were also given an exit score based on the speed at which they unloaded (Table 3.2).

Table 3.1 Description of behaviours recorded during unloading as described by María et al. (2004).

Behaviour	Description
Falls	When any part of the body other than the hooves touches the ground
Reversal	When the animal changes direction to move against the animal flow
Aggression	Agonistic behaviour observed between animals
Mount	When an animal mounts another animal
Balks	When an animal stops completely for more than 10 seconds
Jumps	When an animal jumps
Slips	When an animal looses balance temporarily, interfering with its normal walking
Eliminations	Urination and defecation

Table 3.2 Description of exit score recorded during unloading. Scores were assigned based on the majority of the mob and included half scores.

Score	Description
0	The majority of cattle unloaded slowly in a 4 beat gait (i.e. walking slow)
1	The majority of cattle unloaded swiftly in a 4 beat gait (i.e. walking fast)
2	The majority of the mob exited slowly in a 2 beat gait (i.e. running slow)
3	The majority of cattle exited swiftly in a 2 beat gait (i.e. running fast)

3.2.3 Lairage behaviour

Lairage behaviour was recorded on the afternoon cattle arrived at the plant, and again on the morning of slaughter. Cattle behaviour was observed in lairage pens and occurred no less than one hour after cattle were unloaded to allow them to settle into pens. On the morning of slaughter, observations began from 4:30am, prior to operations and pre-slaughter hide washing commencing. Behaviour was observed from a gantry located above the pens which allowed all animals to be clearly viewed. All observations were made by the same observer, an animal scientist with beef cattle experience. Recording commenced following a period of approximately three minutes of the observer standing in position to prevent immediate behaviour being a result of movement on the gantry and presence of a human. Behaviours were then recorded over a 15 minute period using both scan sampling, with scans every three minutes, and continuous recording. The behaviours recorded using scan sampling were shaking, head down, and laying down (Table 3.3). The wash group was also given a group movement score based on the majority of animals in the group (Table 3.4). The number of cattle drinking, vocalising, mounting and displaying agonistic interactions was recorded continuously over the 15 minute period. Scan behaviours and group movement score were averaged for the recording period and expressed as the percent of the group exhibiting the behaviour, or the average group movement score, respectively. Behaviours recorded continuously throughout the period were expressed as the percent of the group exhibiting the behaviour. Wash groups were also assigned a cleanliness score at the start of the observation period (Table 3.5).

Table 3.3 Description of behaviours observed in lairage using scan sampling techniques.

Behaviour	Description
Shaking	When the animal is rotating vigorously around the medial plane, and movement may include the ears, head, neck and shoulders.
Head down	When the animal's head and neck extend 45° or more below the point of its wither.
Laying	When any point of the animal's sternum is touching the ground. The front legs are usually tucked under on either side of the sternum, and the back legs both extending on the same side of the animal.

Table 3.4 Description of scores used to evaluate group movement. Half scores were used.

Group movement score	Description
0	The majority of cattle were standing stationary and still
1	The majority of cattle were standing stationary, but shuffling their feet, swaying, or fidgeting
2	The majority of cattle were moving around the pen in a 4 beat gait (walking)
3	The majority of cattle were moving around the pen in a 2 beat gait (running)

Table 3.5 Cleanliness score description. Mob cleanliness was recorded during unloading, lairage and on entry to the raceway, and included half scores.

Score	Description
1	The majority of cattle were free from debris and the hair coat could be easily seen all over the body.
2	The majority of cattle were covered lightly in debris, particularly over the legs, belly and rump, however the hair coat beneath the debris could still be seen.
3	The majority of cattle were covered densely in debris, particularly over the legs, belly, rump, back, sides, and shoulder, and the hair coat beneath the debris was not visible.

3.2.4 Pre-slaughter hide washing

Pre-slaughter hide washing was conducted on the morning of slaughter and proceeded in three ordered treatments: lairage washing, high-pressure hosing, and belly washing. The number and duration of each type of wash was recorded (Table 3.6). A water on, water off event was considered one wash, regardless of duration. Water supply to each pen could be turned on and off independently. Cattle were washed in the order they would be presented for slaughter, such that those scheduled to be slaughtered first would be washed first. Lairage washing commenced from 4.30am on the day of slaughter and occurred in lairage pens using untreated water from in-floor sprinklers located in the pens. This was followed by high-pressure hosing where up to two operators would use hand-held high-pressure hoses delivering untreated water to target wash cattle within a wash group. Cattle then moved into a belly wash pen with in-floor sprinklers which used treated water, delivered at a higher pressure compared to lairage washing. All washing treatments applied to a wash group were at the discretion of plant staff. Water temperature and pressure were not recorded.

Table 3.6 Summary of the pre-slaughter hide washing treatments applied to cattle. The mean, minimum, maximum, standard deviation, and coefficient of variation are given for each wash type, and for the total number of washes across types.

	Mean	Min – Max	Standard deviation	Coefficient of variation
		Total washes		
Number	6.1	2 – 13	2.0	33
Duration (mins)	75	5 – 136	30	40
		Lairage washes		
Number	4.0	0 – 7	1.4	39
Duration (mins)	64	0 – 123	29	46
		High-pressure hose washes		
Number	2.0	0 – 7	1.2	74
Duration (mins)	8.3	0 – 32	5.8	70
		Belly washes		
Number	1.0	0 – 2	0.4	42
Duration (mins)	0.9	0 – 10	1.3	147

3.2.5 Carcase grading data

Meat Standards Australia trained graders assessed carcases following active chilling and this grading data was obtained for analysis. Carcases were classified as dark cutters based on pH and meat colour scores (pH >5.71 and/or meat colour >3). Over the period of this study, meat colour score was removed from the MSA grading requirements (30 June 2017) and dark cutting carcases became identified based on pH >5.71 alone. For consistency throughout this study, meat colour >3 remained a criterion for classifying a carcase as a dark cutter. Carcase grading data was then used to identify dark cutting carcases within a wash group, and the percentage of the wash group classified as dark cutters was calculated and used for analysis.

3.2.6 Statistical analysis

Data were analysed using GenStat software (GenStat, 19th Edition, VSN International, Hemel Hempstead, UK) to determine the effect of pre-slaughter factors on dark cutting incidence. Behavioural observations were converted to the percent of the group exhibiting the behaviour for analysis. Linear mixed models were developed to determine the effect of unloading, behaviour, and pre-slaughter hide washing traits on dark cutting incidence. Each factor and behaviour were tested as fixed effects individually. Day killed was included as a random term in all models. Lairage pen (1-25) was also included as a random term for analysis of lairage behaviour and pre-slaughter hide washing traits. The variance accounted for by day killed was consistent across models. A linear mixed model was fit with fixed effects including all traits in each section (unloading, lairage behaviour, pre-slaughter hide washing) and day killed as a random term, as well as pen number for lairage behaviour and pre-slaughter hide washing traits. The variance accounted for by day killed and pen number in these models is reported. Significance was defined as $P<0.05$. The effects of variables on dark cutting incidence are reported as estimated effect, or predicted mean, for the proportion of animals in a wash group classified as dark cutters. Results are presented as mean ± standard error unless stated otherwise.

3.3 Results

Dark cutting incidence was 25% within this study (Table 3.7). Of the 764 dark cutting carcases identified, 756 carcases were dark on both pH and meat colour, and just eight carcases were classified dark based on meat colour score only. A total of 164 wash group replicates were formed from 86 mobs, with some vendors consigning multiple mobs.

Table 3.7 Data summary statistics of cattle observed during this study and Meat Standards Australia carcase grading data.

	Count
Mobs	86
Vendors	62
Animals	3,054
Wash groups	164
Carcases with pH > 5.71	756
Carcases with meat colour > 3	764

3.3.1 Unloading

The most common type of truck used for transportation was semi trailer (n=30), followed by b-double (n=15) and unclassified (n=8). Unloading was not recorded for 20 replicates. B-double, semi-trailers, and unclassified each transported a mean of 49±5, 33±2, and 14±1 head of cattle, respectively. Slipping was the most observed behaviour during unloading with a mean 9.3±1.1% of cattle within a group recorded to slip. This was followed by falls (1.5±0.3%), reversals (0.8±0.2%), jumping (0.6±0.2%), baulking (0.6±0.1%), vocalisation (0.2±0.1%), and mounting (0.05±0.04%). Eliminations and aggressive bouts were not observed during unloading.

Behavioural and environmental factors at the time of unloading had a significant effect on dark cutting incidence (Table 3.8). As the percent of the group slipping or falling during unloading increased, there was an associated decrease in dark cutting incidence. Conversely, a higher incidence of mounting and jumping was associated with a higher dark cutting incidence. Increases in the number of stock on a truck, the time taken to unload, and exit score of the group were also associated with an increase in dark cutting incidence

Table 3.8 The effect of unloading factors on dark cutting incidence. Behaviours are expressed as percent of the group (%) and time is given in minutes. Estimated effects ± standard errors are provided for significant factors (P<0.05). Day killed was included as a random term with an associated variance of 60.6%. Residual variance was 39.4%.

Factor	F pr	Estimated effects ± standard error
Truck number of stock	0.036	0.31 ± 0.15
Truck number of decks	0.282	
Truck type	0.08	
Baulk	0.111	
Vocalisation	0.880	
Falls	0.035	-1.79 ± 0.84
Slips	0.003	-0.86 ± 0.28
Reverse	0.380	
Mounting	0.001	18.5 ± 5.65
Jumping	0.020	2.07 ± 0.88
Exit score	<0.001	14.49 ± 4.31
Unload time total	0.034	1.39 ± 0.65
Unload time per animal	0.227	
Unload ramp	0.968	

3.3.2 Lairage behaviour

Head down was the only lairage behaviour associated with an increase in dark cutting incidence (Table 3.9). For each one percent increase in the proportion of a group scored with head down on the morning of slaughter, dark cutting incidence also increased by 0.33±0.16%. Mounting in lairage pens on the afternoon of arrival tended towards significance (P=0.061).

Table 3.9 The effect of lairage behaviour in afternoon or morning on dark cutting incidence. Behaviours are expressed as percent of the group (%). Estimated effects ± standard errors are provided for significant factors (P<0.05). Day killed and pen were included as random terms. For afternoon behaviours, the variance associated with each random term was: day killed 49.2%, pen 20.8%, residual 30.0%. For morning behaviour, the variance associated with each random term was: day 49.9%, pen 19.1%, residual 31.0%.

Factor	Time	F pr	Predicted mean ± standard error
Drinking	Afternoon	0.228	
	Morning	0.429	
Mounting	Afternoon	0.061	
	Morning	0.889	
Vocalising	Afternoon	0.753	
	Morning	0.839	
Cleanliness	Afternoon	0.321	
	Morning	0.289	
Agonistic	Afternoon	0.762	
	Morning	0.742	
Shaking	Afternoon	0.338	
	Morning	0.339	
Head down	Afternoon	0.193	
	Morning	0.038	0.33 ± 0.16
Laying down	Afternoon	0.637	
	Morning	0.564	
Group movement	Afternoon	0.304	
	Morning	0.762	

3.3.3 Pre-slaughter hide washing

The number and duration of both lairage and high-pressure hose washes had a significant effect on dark cutting incidence (P<0.05, Table 3.10). Each extra lairage wash increased dark cutting incidence by 5.46±2.67%, whilst increasing wash duration was associated with less dark cutting. Increasing the number of high-pressure hose washes also resulted in less dark cutting.

Table 3.10 The effect of lairage washing and high pressure hosing on dark cutting incidence. Collection period (year) was included in each multivariate model, along with the number and duration of each wash type. Estimated effects ± standard errors are provided for significant factors (P<0.05). Day killed and pen were included as random terms and the variance associated with each was: day killed 49.2%, pen 16.1%, residual 34.7%.

Factor	F pr	Estimated effects ± standard error
Lairage washing		
Collection period	0.954	
Wash number	0.043	5.46 ± 2.67
Wash duration (min)	0.041	-0.27 ± 0.13
High pressure hose		
Collection period	0.488	
Wash number	0.030	-3.73 ± 1.70
Wash duration (min)	0.413	

The number and duration of washes within each wash type were correlated, ranging from r=0.22 for belly washes to r=0.86 for lairage washes (Figure 3.1). The strongest correlation (r=0.97) in washing traits was lairage wash duration and total wash duration, reflecting the larger contribution of lairage wash duration to total wash duration (Table 3.6).

The relationship between wash number and duration is explored in 3.4.1.1 The number and duration of washes across each wash type (lairage, high-pressure hose, belly wash) were not correlated (r=<-0.26).

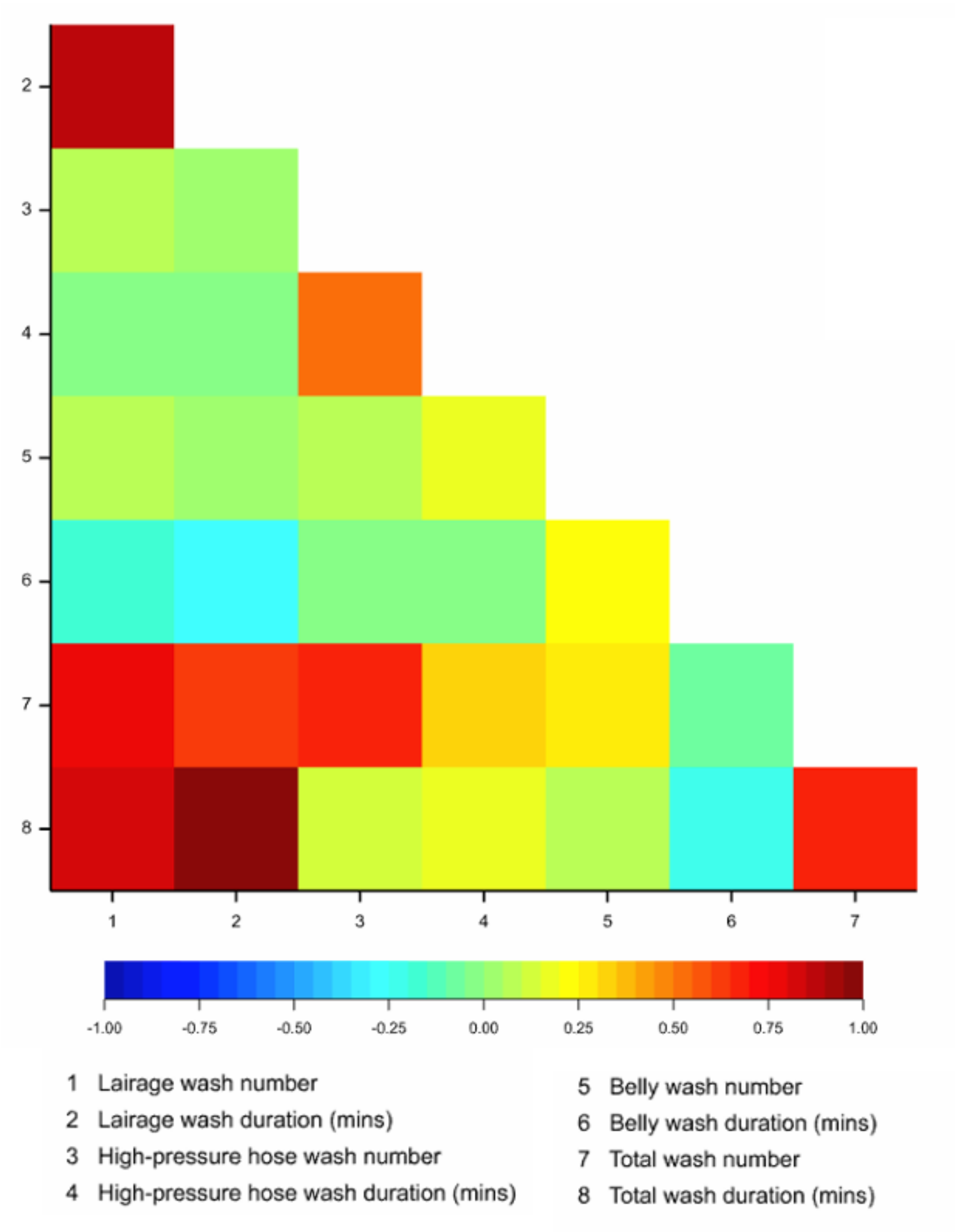


Figure 3.1 Correlations between number and duration of each wash type. Strongly negatively correlated traits are dark blue, strongly positively correlated traits are dark red, and non-correlated traits are green.

Dark cutting is a highly multifactorial problem and this study presents data indicating several components during the pre-slaughter period that have the potential to effect dark cutting incidence in beef. Interpretation of the results from this observational study should have an emphasis on their ability to indicate factors associated with dark cutting incidence that can be managed to reduce its risk. A controlled study would provide more confidence in the absolute size of effect each of the factors reported here have on dark cutting incidence.

3.4 Discussion

Dark cutting incidence in this study was 25%, more than double the Australian national average of 10.8% for pasture finished cattle during the same period (Meat and Livestock Australia 2017). Dark cutting is the greatest contributor to Meat Standards Australia non-compliance (Meat and Livestock Australia 2017) and is a particular problem in pasture finished cattle, especially in the South East region of South Australia, where many cattle in this study originated. The seasonal variation in dark cutting incidence is well reported, with the period from February to June having a significantly high incidence in South Australia (McGilchrist *et al.* 2014). There was also a noticeable peak for this state in dark cutting during July 2015 (Meat and Livestock Australia 2017). Muscle glycogen concentration is influenced by season, with levels at their highest in spring and lowest in winter, respectively (Knee *et al.* 2004). Decreased pasture availability and quality result in lower metabolisable energy intake, resulting in declining animal growth rates and muscle glycogen concentration (Knee *et al.* 2004). Data for this study were collected during June to November due to the greater availability of pasture finished cattle resulting from higher turn off throughout this period. However, despite data collection occurring during a time of typically low incidence (McGilchrist *et al.* 2014), exceptionally high levels of dark cutting were recorded. The high incidence of dark cutting observed in this study might be reflective of regional, on-farm factors linked to this problem (Loudon *et al.* 2018) or unusual seasonal variation in the years sampled, exemplified by the July 2015 peak (Meat and Livestock Australia 2017). In addition to these known factors, this study identified several environmental factors and behaviours throughout the lairage period that significantly impacted dark cutting incidence.

3.4.1 Pre-slaughter hide washing

The various parameters associated with pre-slaughter hide washing of cattle were novel factors investigated during this study and had a significant effect on dark cutting incidence. Both lairage wash number and duration had a significant effect on dark cutting. Each additional wash was associated with an increase in dark cutting incidence of 5.46 ± 2.67%, whilst a longer duration of washing was associated with less dark cutting (Table 3.10). Walker *et al.*(1999) reported lower levels of muscle glucose and lactate concentration in washed cattle compared to unwashed controls. A similar effect was reported by Petersen (1983), where a linear relationship between the number of washes and ultimate pH (pH_u) was found in sheep. In that study, the washing treatment involved sheep swimming the length of a 1.56m bath, either 1, 24, or 48 hours prior to slaughter. Regardless of the point prior to slaughter at which washing occurred, the mean pH_u was greater than 5.7 for sheep that received three or five washes (Petersen 1983). Exercise induced glycogen losses (Jacob *et al.* 2009) may have contributed to the association between ultimate pH and number of washes in that study by Petersen (1983).

The number of high-pressure hose washes also had a significant effect, with more washes being associated with less dark cutting. However, the duration of high-pressure hosing was not significant. It is surprising that wash number had a positive association, increasing dark cutting incidence for one wash type, and a negative association for a subsequent wash type. Lairage washing is the first type of washing cattle receive, conducted in large holding yards early in the pre-slaughter process and may commence several hours before slaughter. In comparison, high-pressure hosing is conducted at a time much closer to slaughter in narrower raceway yards leading to the knocking box and may occur only 30 minutes prior to slaughter. Glycogen mobilised during lairage washing would have sufficient time to be completely metabolised by the time of slaughter, resulting in a higher incidence of dark cutting. However, due to high-pressure hosing occurring much closer to slaughter, any glycogen that is mobilised as a result of stress during this process may not be completely metabolised by the point of slaughter, and thus contribute to pH decline. It is unlikely that high-pressure hosing is a less stressful event than lairage washing, and more likely that the difference in time prior to slaughter at which these procedures occur is impacting these results and thus requires further investigation.

Another possibility for the negative relationship between high-pressure hosing and dark cutting incidence is that not all cattle are impacted by the high-pressure hose. Lairage staff use hand-held high-pressure hoses to target wash cattle during this process, with up to two staff working on one pen of approximately 20 cattle. Thus, whilst all cattle from a wash group will be in the designated high-pressure hosing pen, they may not all receive a wash using the hose if other, dirtier cattle are targeted for washing by staff. However, based on observations throughout collection of data for this experiment, this scenario occurred infrequently and more often than not, all cattle in a group received some high-pressure hosing. Furthermore, there is potential for greater human-animal interactions during high-pressure hosing with staff present and in close proximity to cattle during this procedure. The relationship between human-animal interactions and the stress response is well reported, highlighting the potential for this period to activate the stress response and mobilise glycogen stores, increasing dark cutting incidence (Hemsworth *et al.* 2011). It is likely factors inherent in the nature of this observational study are responsible for the negative relationship recorded between high-pressure hosing and dark cutting incidence, and this deserves further exploration.

The large effect of wash number on dark cutting incidence for both lairage washing and high-pressure hosing suggest it is the water on event causing the response, more so than the continued exposure to the water. Cattle show avoidance behaviours by moving away from stimuli inducing stress (Romeyer and Bouissou 1992), and novelty is a particularly strong stressor (Stephens and Toner 1975; Dantzer and Mormède 1983; Grandin 1997). There is potential for habituation to novel, stress inducing stimuli such as pre-slaughter washing. However, based on the time reported in other studies it is unlikely to occur within the period of exposure during lairage (Kutzer *et al.* 2015).

Although pre-slaughter hide washing is common throughout Australia, this practice has received little consideration as to its effectiveness, or any associated impacts, including effects on beef quality. Literature reporting the effectiveness of pre-slaughter hide washing with water are limited, but those available do not provide a supportive case for the effectiveness of this procedure at controlling carcase hygiene, with some suggesting hide washing increases microbial carcase contamination (Biss and Hathaway 1996; Mies *et al.* 2004; Walia *et al.* 2017). The results of this study indicating the potential for pre-slaughter hide washing to affect dark cutting incidence, and the lack of evidence to demonstrate the effectiveness of this procedure, highlight the need to review pre-slaughter hide washing practices.

3.4.1.1 The correlation between wash number and duration

There was a positive correlation between the number and duration of wash types recorded in this study (lairage, high-pressure hose, belly wash). This relationship is not surprising given an additional wash cannot be applied without inherently increasing duration. Analysis of preliminary data showed lairage wash number had a significant ($p<0.05$) effect on dark cutting incidence when fit in both a univariate analysis and a final multivariate linear model, which included other pre-slaughter factors with a significant effect on dark cutting incidence that were identified in separate precursory univariate analyses (Preston *et al.* 2016). The change in significance between initial analysis (significant in univariate analysis) and the final results presented here (significant in multivariate analysis) could be a result of the large variation associated with day killed or reflect the limitations of this observational study. Day killed accounted for 49.2% of the random variation in the model fitted, indicative of the day-to-day changes in cattle presented for processing, their on-farm and pre-slaughter management, and reflecting the highly multifactorial nature of the dark cutting condition. Additionally, the sensitivity of this analysis may have been limited by underlying bivariate data for dark cutting incidence, with carcases recorded as dark cutters or not. The extent of lactate accumulation or muscle glycogen concentration was not measured and could have provided greater insight, including identification of lower levels of muscle glycogen concentration associated with wash number or duration, but not depleted to the extent that dark cutting occurs.

The relationship between wash number, duration, and dark cutting incidence is reported here following the significant effect these factors were found to have during preliminary analysis. Additionally, literature support that pre-slaughter hide washing is a stressor leading to reduced muscle glycogen stores and high pH (Petersen 1983; Walker *et al.* 1999). Furthermore, the results of this work reported in subsequent chapters demonstrate a relationship between animal behaviour and dark cutting incidence (Chapter 4), and the effect of pre-slaughter hide washing on animal behaviour (Chapter 5), providing support that washing is a stressor related to intrinsic meat quality and animal welfare.

Whilst the wash number and duration within a particular wash type were correlated, there was not a relationship between number or duration across wash types ($r<-0.26$). This could suggest washing treatments were not applied in relation to animal cleanliness, or that their effectiveness was not consistent. If washing was consistently effective at improving visual cleanliness, it would be expected that the number and duration across washing treatments would be related. Alternatively, if washing treatments were applied at random and not based on visual cleanliness, this could also result in a lack of relationship across washing treatments.

3.4.2 Unloading

Another process to which animals bound for slaughter are exposed and that has been recognised as a source of stress is unloading. Unloading has been identified as a less stressful process than loading, but both are more stressful than the transport journey itself (María *et al.* 2004). Several factors and behaviours during this process were associated with dark cutting incidence in the study herein, identifying unloading as an important part of the pre-slaughter process for meat quality.

In the current study an increase in number of stock, mounting, jumping, exit score and total unloading time were associated with increased dark cutting incidence. Stocking density during transport and its effects on cattle physiology and behaviour has been reported. Studies have shown that as stocking density increases, so too does plasma cortisol, glucose, and heart rate, indicating an increase in stress (Tarrant 1989; Honkavaara *et al.* 2003). This is partly due to the behaviour and orientation of cattle being restricted during transport as stocking density increases (Tarrant 1989). In the study herein, truck type tended towards significance (Table 3.8, P = 0.08), suggesting the effect of number of stock on dark cutting incidence was more a factor of the number itself, rather than the type of truck, and larger truck types being able to transport more stock. However, given truck type tended towards significance, this requires further investigation. It is unfortunate that stocking density could not be calculated within this study due to not having access to truck specifics. There was large variation in truck types, especially in the crates of smaller vehicles, and the floor space available to groups due to the way they were penned within a truck varied. Access to this information is an important consideration for future studies.

Both mounting and jumping during unloading have been negatively implicated with animal welfare and correlated with physiological parameters of stress (María *et al.* 2004). It follows that by activating the stress response, these behaviours would also be associated with an increase in dark cutting incidence, as observed in our study. In contrast, Maria *et al.* (2004) did not report an effect on ultimate pH due to these unloading behaviours, and this difference between studies may be due to characteristics of the animals sampled, especially feed type. Exit score and total unloading time were also associated with increased dark cutting incidence. Cafe *et al.* (2011) previously reported higher ultimate pH to be linked with increasing flight speed, and Coombes *et al*. (2014) found a link to increased plasma and muscle lactate but reported no effect of flight speed on muscle glycogen. The difference in effect of flight speed on muscle glycogen and pH in these studies may be due to cattle feed type and initial glycogen availability. Total unloading time was also associated with increased dark cutting incidence, which is surprising given the results of exit score, where a higher score indicating quicker unloading was found to be associated with an increased incidence of dark cutting. It is likely that the effect of unloading time is not at all reflective of exit score, and cattle unloading quicker with a higher exit score, may have taken a greater time in total to unload. Stressed animals may have been less responsive to moving, thus taking longer to unload, or complications with unloading such as jammed gates, may have increased the total time taken and so still represent a stressful experience which would contribute to increased dark cutting incidence.

Falling and slipping during unloading were associated with less dark cutting. Falls occur more often during loading than unloading (María *et al.* 2004) and can be a result of slippery floors, particularly if they lack grooves (Grandin 2007). These behaviours are likely a consequence of unloading environment, rather than due to increased stress on the animal.

3.4.3 Lairage behaviour

Behaviours in this study were recorded in the afternoon after cattle arrived at the plant, and again in the morning prior to slaughter. Diurnal effects on behaviour are reported (Albright and Arave 1997), so it is expected differences between afternoon and morning sampling periods occurred. Variation in the lairage environment in the afternoon and morning are also likely to contribute to these differences. After unloading, cattle are penned within their own mob; no mixing between mobs occurs. However, larger mobs may be penned into smaller groups to accommodate lairage pen size, resulting in regrouping within a mob. Such regrouping results in the need to re-establish social hierarchy, which can cause an increase in agonistic interactions (Brakel and Leis 1976; Schirmann *et al.* 2011) as was observed in this study and which is further impacted by the novel lairage environment (Schirmann *et al.* 2011). Behaviours do not return to baseline levels for five and up to 15 days following regrouping (Kondo *et al.* 1984; Hasegawa *et al.* 1997; Von Keyserlingk *et al.* 2008), indicating behaviour throughout lairage will be affected. The morning of slaughter also presents greater human-animal interactions while ante-mortem vet checks are conducted, and pre-slaughter hide washing commences. The effects of pre-slaughter hide washing on dark cutting are detailed above (Chapter 3), but the possibility for water being used for washing to affect an adjacent pen should also be considered. During data collection for this study it was observed that water from one pen could enter an adjacent pen, and so even when a group of animals is not being washed, then may still be affected by washing occurring in neighbouring pens.

The most commonly observed behaviours during this study were vocalisation and head down, with all other behaviours displayed by no more than 3% of a group. Head down on the morning of slaughter was the only lairage behaviour associated with dark cutting incidence. As head down increased, so too did dark cutting incidence (Table 3.9), suggesting this behaviour is linked to activation of the stress response, whereby glycogen breakdown is initiated. This is further supported by the increase in plasma cortisol concentration linked to head down behaviour (Hemsworth *et al.* 2011). Vocalisations were not associated with dark cutting incidence in this study, although they have previously been linked to increased cortisol concentration (Hemsworth *et al.* 2011). The type of vocalisation was not recorded during this study and rather than an indicator of stress (Grandin 1998), some vocalisations may have been a form of communication and it would be useful to discriminate between the two in future studies. Mounting on the afternoon of arrival tended towards significance (Table 3.9, $P = 0.06$) and is associated with decreased muscle glycogen and dark cutting (Kenny and Tarrant 1988)), the initiation of which is known to occur as a result of stress (Veissier and Boissy 2007). Regrouping on the afternoon of arrival is a likely cause of this stress, and has been linked to increased mounting behaviour (Albright and Arave 1997). Resting behaviours including laying down and drinking were not associated with dark cutting incidence as expected. Laying down was observed less on the afternoon of arrival than on the morning of slaughter; this behaviour is known to decrease following regrouping (Von Keyserlingk *et al.* 2008) and follows diurnal rhythms.

3.5 Conclusion

The results of this study highlight the importance of the lairage period on overall beef quality. Several behaviours and environmental factors during the pre-slaughter period were related to an increased incidence of dark cutting beef, including those occurring during unloading, lairage yarding, and throughout pre-slaughter hide washing. This study supported our hypothesis and identified the potential to minimise the risk of dark cutting through enhanced management during the pre-slaughter period. This observational study has provided preliminary data to better guide future work in lairage studies, and identified key elements of lairage which can be managed by processors to minimise the risk of dark cutting, particularly in already vulnerable animals such as pasture finished cattle.

Chapter 4 | Pre-slaughter factors affecting behaviour in pasture finished beef cattle

4.1 Introduction

Animal welfare is becoming of increasing importance to modern consumers (Purslow 2017), whom also consider eating quality when making repeat purchasing decisions (Egan *et al.* 2001). Poor animal welfare has the potential to limit meat quality (Wigham *et al.* 2018), with lesser quality products incurring financial penalty (Jose *et al.* 2015). Therefore, it is in the best interests of producers, processors, consumers and animals for good animal welfare to be maintained as a priority.

The Australian Animal Welfare Standards and Guidelines for Cattle (2016) define animal welfare as “the state of an animal and how well it is coping with the conditions in which it lives.” A behavioural change commonly occurs when welfare is challenged and an animal is unable to cope with its surroundings (Barnett and Hemsworth 1990). Stimuli causing stress result in avoidance behaviours exhibited as part of the flight response in an animal’s attempts to remove the stressor from its environment (Romeyer and Bouissou 1992). Behaviour is thus a useful, non-invasive output based tool for assessing animal welfare, particularly in comparison to other techniques like blood sampling (Zavy *et al.* 1992).

The effects of poor welfare on meat quality are well described (Wigham *et al.* 2018). Stimuli inducing stress, be they real or perceived, initiate the stress response through activation of the hypothalamic-pituitary-adrenal (HPA) axis, releasing cortisol which in turn stimulates glycogenolysis (Sapolsky *et al.* 2000; Veissier and Boissy 2007; Ferguson and Warner 2008). Consequently, insufficient levels of muscle glycogen at slaughter result in dark cutting beef (Tarrant 1989), one of the greatest meat quality challenges for pasture finished cattle.

Cattle behaviours have been linked to physiological parameters, including blood cortisol concentration, which increases in response to stress. Specifically, head down and vocalisations during lairage have both been linked to increased cortisol concentration (Dunn 1990; Hemsworth *et al.* 2011). Behaviours during loading and unloading are also related to plasma cortisol, lactate, and creatine kinase concentration (María *et al.* 2004). Slipping and falling were included among these loading and unloading behaviours and are considered indicators of stress when observed during lairage handling (Grandin 2000).

Abattoir lairages provide places for animals to rest prior to slaughter and recover from the stress of their transport journey. However, lairage is a novel environment with increased human-animal interactions, both of which are stressful experiences (Grandin 1997; Hemsworth *et al.* 2011). Additionally, cattle are kept off feed starting approximately 12 hours prior to transport and continuing throughout the lairage period. Thus, if stressors initiate glycogen breakdown during this time muscle glycogen stores cannot be recovered before slaughter, making dark cutting more likely (Tarrant 1989).

Lairage is a key aspect of beef production and while representing only a small component of an animal's life, it can pose many challenges for animal welfare with lasting effects on meat quality. The aim of this study was to investigate factors in lairage that affect animal behaviour, used as an indicator of welfare. We hypothesise that lairage provides many novel stimuli that are perceived by cattle as stressors, which will be reflected in animal behaviour.

4.2 Materials and methods

Data for this observational cohort study were collected as described in Chapter 3 and a brief summary is provided here. Data were collected between June 2015 and November 2017 over 21 kill days using certified pasture-finished, Meat Standards Australia graded commercial mobs selected randomly from those scheduled to be processed at a large Southern Australian abattoir. This project was approved by The University of Adelaide Animal Ethics Committee (Approval number S-2016-096).

4.2.1 Animal management

A total 3,054 cattle consigned as 86 mobs from 62 different vendors were included in this study. Cattle arrived at the processor the day prior to or morning of slaughter where they were unloaded and then housed in lairage pens. Mixing within mobs occurred where larger mobs had to be separated due to pen holding capacity, but no mixing between mobs occurred. Once penned in lairage, cattle remained in these groups which formed the unit of replication for this study (wash group). Cattle were washed to remove contaminants from the hide in the morning prior to slaughter, which was conducted by captive bolt gun stun followed by exsanguination.

4.2.2 Unloading

Animal behaviour was observed during unloading on one of two ramps. During 2015 a single ramp (old ramp) adjustable in height which allowed unloading from the rear was used.

This ramp had concrete flooring with square tubing slats and was only wide enough that one animal could descend at a time, unable to pass the animal in front of it. This ramp ceased operation following the installation of an additional ramp (new ramp) which allowed rear and side unloading to fixed top and bottom deck ramps. These ramps both had grooved concrete flooring and were wide enough to allow one animal to pass another while descending. During unloading the behaviours baulk, vocalisation, fall, slip, reverse, mounting, jumping, exit score, total unloading time, and unloading time per animal were record (Table 3.1). The type of truck, including number of decks and stock carried, were also recorded.

4.2.3 Lairage behaviour

Cattle were observed in their wash groups while in lairage and their behaviour recorded on the afternoon of arrival and the morning of slaughter. All observations were made by the same observer, an animal scientist with beef cattle experience. Observations were made from the lairage gantry over a 15-minute period. Wash groups were first scored for cleanliness (Table 3.5). Shaking, head down, laying down and group movement were recorded using scan sampling while drinking, mounting and agonistic interactions were recorded continuously (Table 3.3 and Table 3.4). Behaviours recorded using scan sampling were averaged for the recording period from which the percent of the group exhibiting the behaviour was calculated. Behaviours recorded continuously were combined and expressed as the percent of the group exhibiting the behaviour.

4.2.4 Pre-slaughter hide washing

Cattle were washed on the morning of slaughter using three ordered treatments: lairage washing, high-pressure hosing, and belly washing. Water temperature and pressure were not recorded, and all treatments were applied at the discretion of abattoir staff. The number and duration of each wash type was recorded, with a water on, water off event considered one wash. Cattle were washed in wash groups in the order they would be presented for slaughter. Lairage washing commenced from 4:30am using in-floor sprinklers located in lairage pens. High-pressure hosing followed, where up to two operators used hand-held high-pressure hoses to target wash cattle. Finally, cattle were washed in a belly wash pen with water delivered similar to lairage washing but at a higher pressure.

4.2.5 Washing behaviour

Animal behaviour was observed from the lairage gantry during high-pressure hosing. The number of animals in a wash group observed mounting and vocalising were recorded during high-pressure hosing in both the designated pen and the belly wash pen when the high-pressure hose was used.

4.2.6 Statistical analysis

Data were analysed using GenStat software (GenStat, 19th Edition, VSN International, Hemel Hempstead, UK) to determine the effect of pre-slaughter factors on animal behaviour. Behavioural observations were converted to the percent of the group exhibiting the behaviour for analysis. Linear mixed models were developed to determine the effect of unloading ramp (old, new), time of day (afternoon, morning), and wash number and duration (lairage wash, high-pressure hose) on animal behaviour. Behaviours were fit as response variates individually for each factor. Day killed was included as a random effect in all models. The interaction between day killed and time of day was also added to the random effects for models investigating time of day effects on lairage behaviour. Significance was defined as $P<0.05$. The effects of factors on animal behaviour are reported as estimated effects or predicted means.

Results are presented as mean ± standard error unless stated otherwise. The relationship between all behaviours was also investigated using a correlation plot.

4.3 Results

4.3.1 Unloading

Slipping was observed in the greatest proportion of a group on both the old and new ramp during unloading (Figure 4.1). Falling increased from 0.6% on the old ramp to 3.1% on the new ramp, while reversing decreased from 1.4% on the old ramp to 0.3% on the new ramp. Baulking did not change between ramps. Jumping and mounting were only observed on the old ramp, and cattle only vocalised only on the new ramp. Vocalising and mounting were the least frequently observed behaviours, each only recorded in one group.

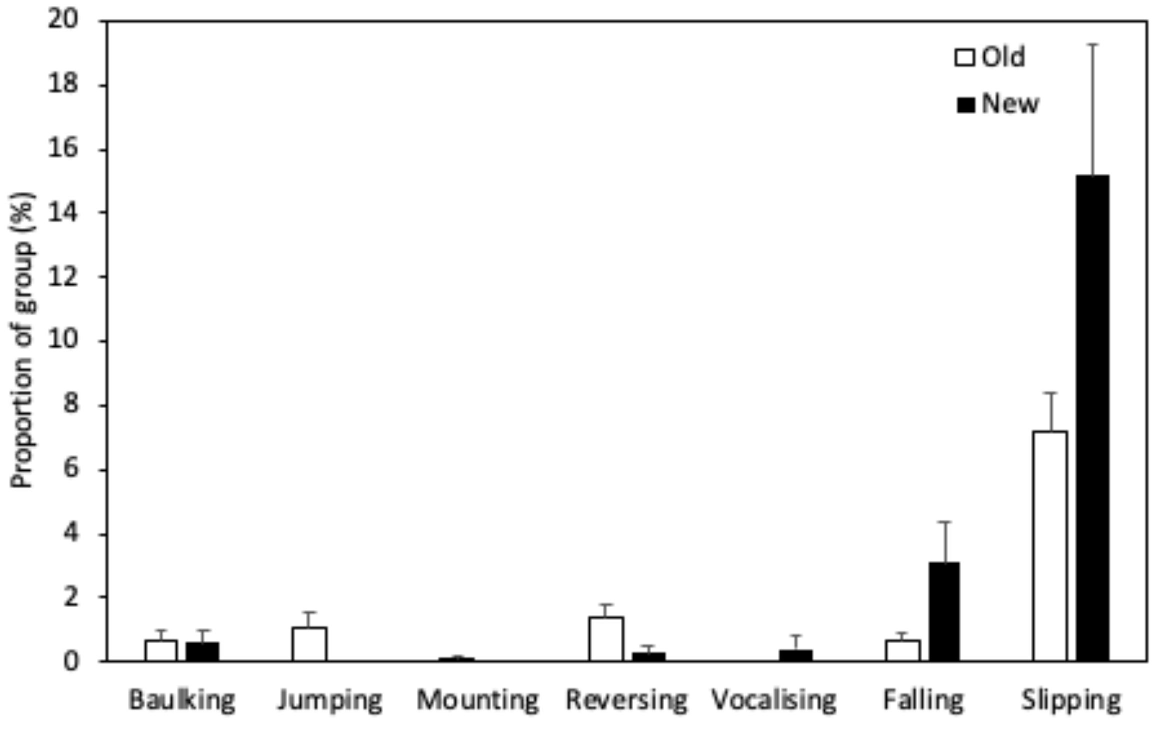


Figure 4.1 The mean ± standard error of the proportion of a group exhibiting a behaviour during unloading. Old and new ramp behaviour observations are separated.

Unloading ramp did not have a significant effect on any unloading factors or behaviours (Table 4.1). Reversing, jumping and number of stock neared significance with P-values of 0.074, 0.074, and 0.081, respectively. Variance associated with day killed was variable, ranging from 0-77.0%.

Table 4.1 The effect of ramp (new or old) on unloading traits and behaviours. Behaviours are expressed as percent of the group (%) and time is given in minutes. The mean ± standard error and significance are provided. Day killed was included as a random factor and the variance it accounts for is given.

	F pr	Old ramp	New ramp	Day variance (%)
Number of stock on truck	0.081	29.4 ± 2.4	43.4 ± 4.2	77.0
Unload time total	0.503	5.3 ± 0.5	6.4 ±1.0	10.5
Unload time per animal	0.136	0.2 ± 0.01	0.1 ± 0.02	0.2
Baulk	0.882	0.7 ± 0.3	0.6 ± 0.3	0
Vocalisation	0.157	0.0 ± 0.0	0.4 ± 0.4	33.4
Fall	0.158	0.6 ± 0.3	3.1 ± 1.2	30.7
Slip	0.322	7.2 ± 1.2	15.2 ± 4.0	57.7
Reverse	0.074	1.4 ± 0.4	0.3 ± 0.2	0
Mount	0.448	0.1 ± 0.1	0.0 ± 0.0	0
Jump	0.074	1.1 ± 0.5	0.0 ± 0.0	0

4.3.2 Lairage

Vocalising was observed on average among a higher proportion of a group than any other behaviour during the afternoon of arrival and morning of slaughter (Figure 4.2). More vocalisations were recorded in the morning (31.2±3.3%) than in the afternoon (20.2±2.0%). Head down was the next most observed behaviour amongst groups, at 14.8±1.2% to 16.8±0.9% in the morning and afternoon, respectively. For all other behaviours recorded in lairage, the average incidence was less than 3.0% of the group observed exhibiting each behaviour. Shaking and laying down increased in the morning compared to the afternoon while drinking and agonistic interactions decreased.

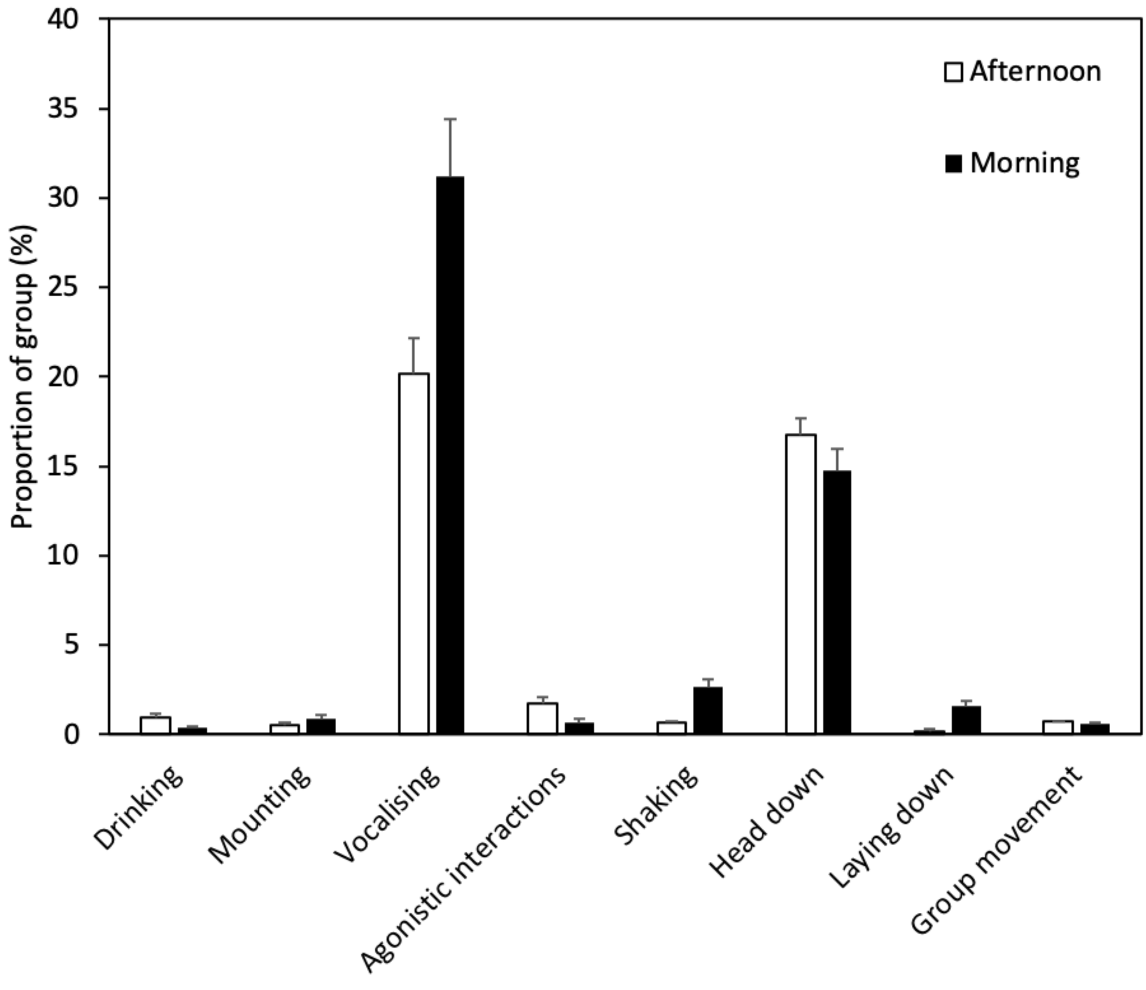


Figure 4.2 The mean ± standard error of the proportion of a group exhibiting a behaviour during lairage. Afternoon of arrival and morning of slaughter behaviour observations are separated.

Time of day had a significant effect on vocalising, agonistic interactions, shaking, head down, and laying behaviours (P<0.05; Table 4.2). Vocalising, shaking, and laying down behaviours increased in the morning while agonistic interactions and head down behaviour had a higher predicted mean on the afternoon of arrival. For all behaviours, the majority of variance was unexplained with residual variance ranging from 71.9-94.4% of the random variance. For the random variance components, half the behaviours had more variance associated with day killed, and half with the interaction between day killed and time of day.

Table 4.2 The effect of time of day (afternoon, pm or morning, am) on lairage pen behaviours. Behaviours are expressed as percent of the group (%). The predicted mean ± standard error and significance are provided. Day killed (between day - B) and the interaction between day killed and time of day (within day - W) were included as random factors and the variance they account for is given.

Response variate	F pr.	Predicted mean (%)	Random variance (%)	Residual variance (%)
Drinking	0.189		B: 0.0* W: 14.9	85.1
Mounting	0.382		B: 0.0* W: 20.6	79.4
Vocalising	0.014	pm: 19.7 ± 3.8 am: 30.9 ± 3.8	B: 14.3 W: 2.8	82.9
Agonistic	0.007	pm: 1.8 ± 0.3 am: 0.7 ±0.3	B: 7.0 W: 0.0*	93.0
Shaking	0.002	pm: 0.6 ±0.4 am: 2.6 ± 0.4	B: 2.4 W: 2.7	94.9
Head down	0.045	pm: 15.8 ±1.7 am: 13.5 ±1.7	B: 27.3 W: 0.0*	72.7
Laying	0.007	pm: 0.1 ± 0.3 am: 1.5 ± 0.3	B: 0.8 W: 13.4	85.7
Group movement	0.157		B: 18.3 W: 9.8	71.9

* indicates a negative variance component when the model was run with both random effects. Significance, means and remaining variance components reported are those obtained when the model was re run without the negative variance component factor.

4.3.3 Pre-slaughter hide washing

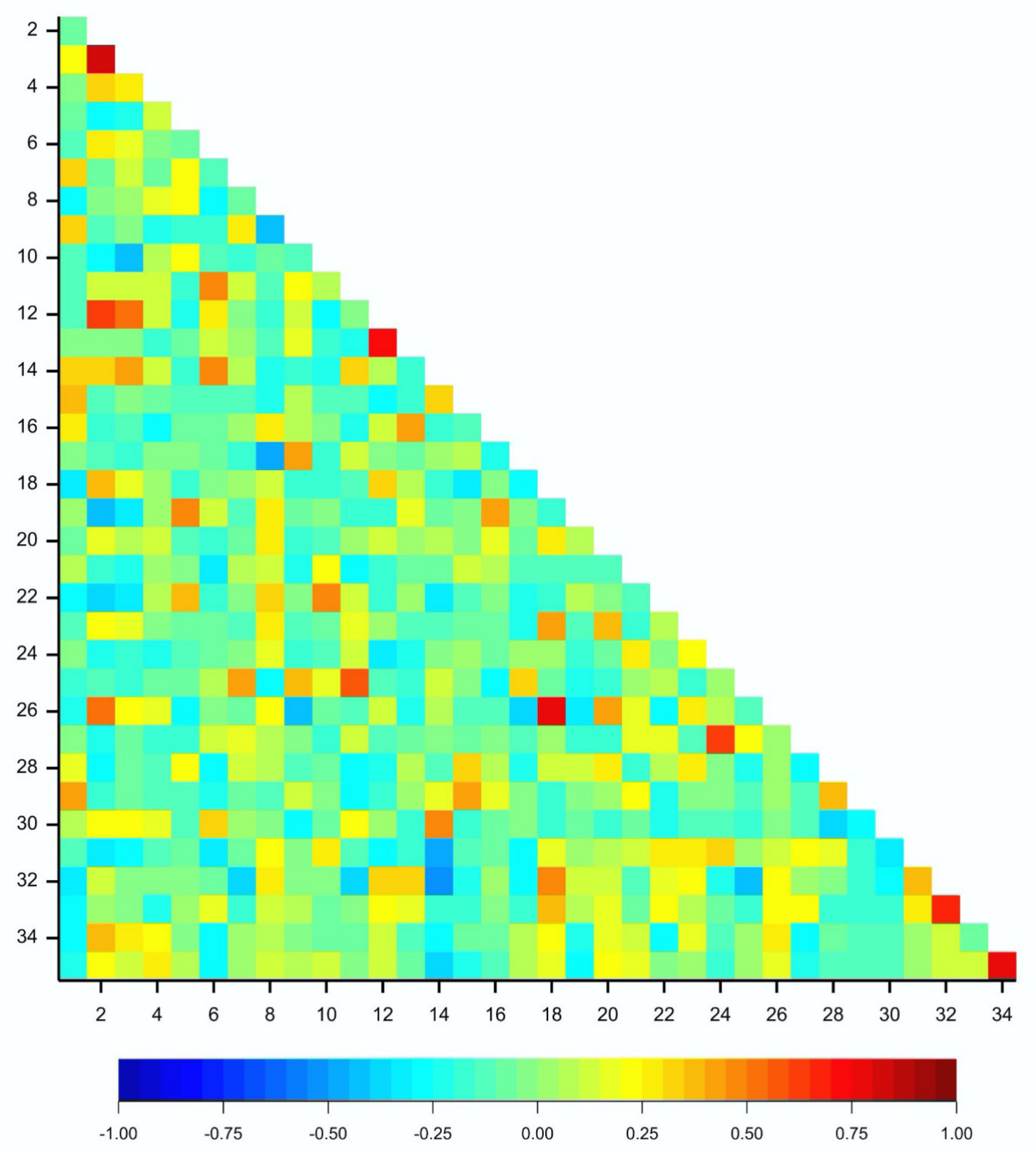
Pre-slaughter hide washing had a significant effect on the proportion of a group vocalising and mounting during high-pressure hosing (Table 4.3). High-pressure hose duration increased vocalisations, and high-pressure hose wash number and duration increased mounting behaviour. For most traits the residual variance, representing that between mobs on a given day, accounted for 94.3-97.1% of the variation. However, for the effect of high-pressure hose duration on vocalisation, 46.7% of the variation was associated with day killed.

Table 4.3 The effect of wash number and duration on the proportion of a group observed vocalising and mounting during high-pressure hosing. The estimated effect ± standard error are provided for significant variables (P<0.05).

Response variate	Explanatory variable	F pr.	Estimated effect (%)	Day variance (%)	Residual variance (%)
Vocalisation	Lairage wash number	0.584		3.7	96.3
	Lairage wash duration (mins)	0.802		2.9	97.1
	High-pressure hose wash number	0.129		3.5	96.5
	High-pressure hose duration (mins)	<0.001	0.1 ± 0.01	46.7	53.3
Mounting	Lairage wash number	0.579		5.7	94.3
	Lairage wash duration (mins)	0.956		5.3	94.7
	High-pressure hose wash number	<0.001	0.4 ± 0.1	4.6	95.4
	High-pressure hose duration (mins)	<0.001	0.1 ± 0.02	4.4	95.6

4.3.3 Correlation between pre-slaughter behaviours

Correlations between behaviours were evident both within and between the main areas where recording occurred (i.e., unloading, lairage, pre-slaughter hide washing; Figure 4.3). At unloading, total unloading time was correlated with: the number of stock on the truck (r=0.62), the number of decks on the truck (r=0.51), as well as the unloading time per animal (r=0.74). Lairage cleanliness on the afternoon of arrival and morning of slaughter were correlated (r=0.78), with 64.9% of wash groups scored on both the afternoon and morning receiving the same score and only 20.2% of groups scoring higher (dirtier) in the morning compared to the afternoon. Agonistic interactions and mounting observed on the morning of slaughter in lairage were also correlated (r=0.65). Wash number and duration for both lairage (r=0.69) and high-pressure hosing (r=0.78) were correlated. Correlations between traits were also observed across areas of lairage, including number of stock on truck and lairage cleanliness on the morning of slaughter (r=0.51), and unloading exit score and vocalisations on the morning of slaughter (r=0.60). The only negative correlation was between unloading ramp and lairage wash number (r =-0.53). Remaining interactions not specified here had an r value below 0.50 for both positive and negative correlations.



1 Dark cutting (%)
2 Unload – truck number of stock
3 Unload – truck number of decks
4 Unload – truck type
5 Unload - baulk
6 Unload - vocal
7 Unload - fall
8 Unload - slip
9 Unload - reverse
10 Unload - jump
11 Unload – flight score
12 Unload – total time (mins)
13 Unload – time per animal (mins)
14 Unload - ramp
15 Lairage pm drinking
16 Lairage pm mounting
17 Lairage pm vocalising
18 Lairage pm cleanliness
19 Lairage pm agonistic
20 Lairage pm shaking
21 Lairage pm head down
22 Lairage pm group movement
23 Lairage am drinking
24 Lairage am mounting
25 Lairage am vocalising
26 Lairage am cleanliness
27 Lairage am agonistic
28 Lairage am shaking
29 Lairage am head down
30 Lairage am laying
31 Lairage am group movement
32 Lairage wash number
33 Lairage wash duration (mins)
34 High-pressure hose wash number
35 High-pressure hose wash duration (mins)

Figure 4.3 Correlation of unloading traits, lairage behaviours, and pre-slaughter hide washing treatments. Colours are derived from regression coefficients such that strongly negatively correlated traits are dark blue, strongly positively correlated traits are dark red, and non-correlated traits are green.

4.4 Discussion

This study recorded beef cattle behaviours which indicate several novel stimuli encountered in Australian lairages impact welfare through initiation of the stress response. Behaviour during unloading, lairage, and pre-slaughter hide washing procedures were observed, with washing practices and time of day having the greatest effect on animal behaviour.

4.4.1 Pre-slaughter hide washing

High-pressure hose duration increased both vocalisations and mounting, the latter which was also increased by the number of high-pressure hose washes (Table 4.3). This suggests this washing technique has an immediate effect on behaviour, and that the longer the duration, the more adverse it becomes for the animal. A study investigating acclimatisation of cattle to transport stress demonstrated an increase in adrenocorticotropic hormone (ACTH) and cortisol following one and two hours of transport, respectively (Knights and Smith 2007). While markers in that study remained high during the transport journey they did not continue to rise after two hours, suggesting acclimatisation to acute stressors occurs after this point through either a change in the animal's perception, or the biochemical response, following prolonged exposure. High-pressure hosing lasted up to 32 minutes in the study herein, falling during the period of increased ACTH and cortisol concentration reported by Knights and Smith (2007), suggesting the peak hormonal response to high-pressure hosing has not been reached and that increased exposure was still resulting in an increased effect on stress, observed in behaviour. The response occurring may have varied between individual animals in a wash group, especially if high-pressure hose operators continuously targeted specific animals over others during the process.

Furthermore, lairage washing did not affect behaviours observed during high-pressure hosing, despite being identified as a source of stress affecting meat quality (Table 3.10) and behaviour (Table 5.3). This suggests that behavioural changes which might have resulted from lairage washing are not long lasting (but can remain for up to 10 minutes post cessation of washing, Chapter 5), at least for vocalisation and mounting behaviour. Lairage washing was conducted prior to high-pressure hosing and lasted from 5-135 minutes, the upper extent of which fits within the time period where hormonal responses to stress reach their peak (Knights and Smith 2007). This further supports the abovementioned theory that acclimatisation begins to occur with either the animal's perception, or biochemical response to continued exposure changing over time.

After accounting for high-pressure hose duration, 46.7% of variation in vocalisation was associated with day killed, much higher than the 2.9-5.7% variation attributed to day killed for all other comparable washing traits (Table 4.3). This large effect could be a result of differences in individual high-pressure hose operators and their technique across days. A range of different operators were observed using the high-pressure during this study and a difference in the way they proceeded with washing were noticed by the observer. Another explanation is that very vocal mobs were processed together, which may have resulted from an increase in vocalisations between animals signalling a stressor in their environment to conspecifics (Rushen 2008). The relationships between washing practices and animal behaviours will be explored in detail in Chapter 5.

4.4.2 Lairage

A significant difference between morning and afternoon was observed for the behaviours vocalising, agonistic interactions, shaking, head down, and laying down in lairage.

This may be a result of the diurnal nature of some behaviours or differences in the lairage environment eliciting these behaviours during each time point. Additionally, some level of acclimation to the lairage environment may have occurred and impacted these results.

The behaviours observed among the highest portion of a wash group during lairage on the afternoon of arrival and morning of slaughter were vocalising and head down, both which occur in response to stress (Dunn 1990; Grandin 1998; Hemsworth *et al.* 2011). These behaviours were observed in a much greater portion of a group compared to all other behaviours, which were only observed in up to 5% of a group at one time (Figure 4.2). The larger number of animals exhibiting these behaviours at the same recording point suggest they are occurring in response to environmental stimuli affecting the whole group, rather than an individual animal expressing itself through behaviour.

Cattle vocalisations are reflective of handling practices and reliably indicate stress (Warriss *et al.* 1994; Grandin 1998). The incidence of vocalisation among cattle at slaughter varies; an average of 3% of animals are observed to vocalise during handling between the crowd pen and the restrainer, with <1% of cattle vocalising at better performing plants and up to 17% vocalising in more poorly performing plants (Grandin 2000). The incidence of vocalisation in the study herein far exceeds these levels (Figure 4.2), suggesting a more stressful environment given vocalisations occur in response to adverse events (Grandin 1998). However, the comparative increase in vocalisations in our study could also be due to recording this behaviour in lairage pens, rather than between the crowding pen and point of slaughter as in the study by Grandin (2000). Vocalisation incidence differed significantly between the afternoon and morning, suggesting morning was a more stressful period (Table 4.2). An increase in vocalisations in the morning could result from the different procedures occurring during this time, including ante-mortem inspection by veterinarians which rouses animals, and the commencement of washing procedures. Stress could thus result from increased human-animal interactions (Hemsworth *et al.* 2011), auditory cues from animals that might be signalling distress (Rushen 2008) because of their own perceptions and environment, or from washing practices impacting animals in pens adjacent to those where washing is occurring. The type of vocalisation was not recorded during this study and may have been in response to the novel environment, rather than in response to a stressor (Rushen 2008). However, if vocalisations were in response to the novel lairage environment, it is likely their incidence would have been higher during the afternoon when first exposed to the environment.

Agonistic interactions and head down were significantly higher on the afternoon of arrival than on the morning of slaughter (Table 4.2). Following transport, larger mobs are separated into smaller groups for penning in lairage resulting in group restructure and mixing, which may initiate conflict behaviours in order for social hierarchy to be re-established. In new surroundings, cattle initially spend time exploring their environment and although aggression between cattle is rare (Rushen 2008), confinement does result in an increase in agonistic interactions (O'Connell *et al.* 1989). The increased head down behaviour may follow as a result of this, or be in response to water coming into the pen from adjacent yards of stock being processed late in the day. Whilst morning and afternoon were statistically different, there was still a high portion of a group scored head down at both times, suggesting both are stressful periods. The lower portion observed head down on the morning of slaughter could indicate that the environment, although still stressful, has become more predictable (Greiveldinger *et al.* 2009), or could reflect the increase in other behaviours during this period (vocalising, shaking, laying down).

Shaking was significantly higher in the morning (Table 4.2), a time when pre-slaughter hide washing may have commenced in pens adjacent to those being observed. Shaking increases in response to discomfort (Vickers *et al.* 2005; Park *et al.* 2017) which may have resulted from water affecting the animal's head. Water from surrounding pens could have reached animals in the pens being observed, given the fences between yards were not solid. Laying down also increased in the morning (Table 4.2) which could be due to the diurnal pattern of this behaviour (Kilgour 2012) coupled with an increase in synchrony of lying occurring in the morning compared to the middle of the day (Stoye *et al.* 2012). Lying is typically indicative of rest (Kilgour 2012) and while this behaviour increased during the morning, it only occurred in a very small proportion of the group (1.5%, Table 4.2). This suggests aversive stimuli may be resulting in asynchrony of this behaviour (Stoye *et al.* 2012) and further supports that this time in lairage is still perceived as stressful by animals.

Drinking and mounting were not affected by time of day. Factors influencing cattle drinking include feed type and intake, the availability and quality of water sources, and ambient temperature (Albright and Arave 1997). Drinking follows a diurnal pattern which can peak at sunrise and sunset (Albright and Arave 1997), thus a difference in time of day was not expected for this behaviour. Although not significantly different, less cattle were observed drinking in the morning, which may have been due to some observations commencing before sunrise. Mounting behaviour was not significantly different, suggesting both morning and afternoon provide challenging times for cattle resulting in this behaviour. Mounting in the afternoon may be related to the increase in agonistic interactions during this period occurring as a result of regrouping or experiencing a novel environment, as discussed above. Events occurring in the morning which could result in mounting include the commencement of pre-slaughter washing and the rousing of cattle to allow antemortem inspections to be conducted.

4.4.3 Unloading

Loading and unloading are a necessary component of beef production and a more stressful part of transport when compared to the journey itself (María *et al.* 2004). Both the old and new ramp used during this study meet the recommendations set out in AS5340:2020 (Standards Australia 2020). Ramp (new or old) did not have a significant effect on any unloading behaviours ($P>0.05$, Table 4.1), suggesting the behaviours observed are more indicative of factors other than the unloading facilities, such as individual animal temperament, on-farm management, and weather.

Slipping was observed among the highest proportion of the group across all behaviours for both the old and new ramp (Table 4.1). The proportion of the group slipping on the new ramp (15.2%) was almost double that observed on the old ramp (7.2%), and in both cases higher than the 4% incidence reported during unloading in other studies (Miranda-de la Lama *et al.* 2012). The square tubing slats on the old unloading ramp may have contributed to the lower slipping incidence when compared to the new ramp. The variance accounted for by day killed was high at 57.7% for this behaviour and may be indicative of a large effect of external factors impacting this behaviour, in comparison to the facilities themselves. Weather events such as rain may have resulted in more slipping on a given day with effects on surfaces potentially lasting into the following day, and such events may have impacted animal behaviour.

A lower proportion of animals were observed reversing on the new ramp (0.3%) when compared to the old ramp (1.4%, Table 4.1). The greater width of the new unloading ramp provides ample room for multiple animals to descend at the same time, and room for animals to easily turn and change direction. Any animals initially exiting the truck in a backwards position would thus be able to right themselves immediately and descend the ramp in a forward facing gait.

Jumping and mounting behaviours were only observed on the old ramp and may have been a result of difference in facility design. The narrow width of the old ramp may have resulted in animals mounting if unable to pass the animal immediately in front during descent. This could also result if animals further down the ramp were baulking while drivers were moving stock in the crate to continue unloading. However, mounting was only observed in one group and more observations would be required to allow proper interpretation. The observer noted almost all jumps occurred at the bottom of the ramp, such that cattle jumped from the ramp to the base of the ramp or the pen into which they were unloading. This may have resulted from the incline or surface of the ramp. However, given most jumps occurred at the same location, it is also possible something in the animals visual field resulted in jumping. For example, the metal grate at the bottom of the ramp used to prevent erosion at this high use area.

Day killed accounted for only a small proportion of total variance (Table 4.1) for all unloading factors, except number of stock and slipping. A high day variance was recorded for the number of stock on a truck (77.0%, Table 4.1), which is likely related to the cyclic nature of cattle production in Australia. In times of peak supply such as drought, larger mobs will be consigned. In comparison, cattle are in short supply when producers only turn off a small number of stock, such as the time following drought when breeding and growing herds becomes a priority.

4.4.4 Correlations

Correlations were observed between behaviours and environmental factors occurring both within and between similar periods of recording (i.e. unloading, lairage, washing).

4.4.4.1 Pre-slaughter hide washing

Wash number and duration were correlated for both lairage washing and high-pressure hosing. This indicates most washes of each type lasted a similar length of time, rather than being comprised of a variation of short washes mixed with long washes. Lairage washing was connected to a timing system with an automatic off and each wash lasted an average 18 minutes. Thus, most washes in lairage lasted this duration unless interrupted, such as when staff might stop water mid wash to move cattle forward to the next stage of processing. Chapter 3 also provides a detailed discussion of the relationship between lairage wash number and time (3.4.1.1). Although high-pressure hosing was conducted manually and not on a timing system, this procedure tended to last for as long as it took the group ahead of those being washed to be processed. Given the consistency of chain speed and processing, time generally only varied with wash group size when smaller groups were processed.

4.4.4.2 Lairage

Lairage cleanliness on the afternoon of arrival and morning of slaughter were correlated, indicating animals tend to remain in the same cleanliness condition in which they arrived. An increase in stocking density (Small and Buncic 2009) and time spent in lairage (Grau and Smith 1974) are related to a deterioration in the visual cleanliness of cattle, which is likely a result of a more confined space increasing contact between animals (Small and Buncic 2009), especially over a longer period. However, in this study cleanliness remained unchanged between the afternoon of arrival and morning of slaughter for 64.9% of wash groups. Visual cleanliness of cattle is significantly affected by faecal consistency, where cattle with firmer faeces are cleaner (Hughes 2001; Ward *et al.* 2002). Cattle in this study were pasture-finished and thus likely had a diet high in fibre which results in firmer faecal consistency (Hughes 2001) increasing the difficulty for faecal material to be spread. These results suggest time spent in lairage does not tend to increase the dirtiness of pasture finished cattle, rather their cleanliness is more reflective of on-farm management and transport, resulting in the condition in which they arrive. Stocking density in lairage pens was not recorded in this study and may have contributed to lairage cleanliness; if stocking density was low, less contacts between animals may have occurred, thus reducing opportunities for animal-animal transmission of contaminants.

Vocalisations on the morning of slaughter were positively correlated with exit score at unloading, both of which are associated with stress (Grandin 1998; Vetters *et al.* 2013). Exit score provides an indication of temperament (Vetters *et al.* 2013) where animals with a higher (faster) exit score have a more excitable temperament and greater basal cortisol concentrations (Burdick *et al.* 2011). Additionally, vocalisations are used to communicate with conspecifics (Watts and Stookey 2000) and are reliable indicators of stress (Warriss *et al.* 1994; Grandin 1998). Although the type of vocalisation was not recorded in this study, the positive correlation with exit score supports vocalisations on the morning of slaughter occurring in response to stress, which also indicates consistency in response based on the animal's temperament. Furthermore, agonistic interactions and mounting behaviour on the morning of slaughter were correlated, both of which also occur in response to stress (Tennessen *et al.* 1985; Kenny and Tarrant 1987). The relationships between these behaviours' indicative of stress on the morning of slaughter suggest cattle are exposed to significant aversive stimuli during this period.

4.4.4.3 Unloading

The total time taken to unload was correlated with: the number of animals on the truck, the number of decks on the truck, and the unloading time per animal (Figure 4.3). This is an expected result; as the number of decks on a truck increases, the number of animals that can be carried also increases, and thus so does the total time taken for all animals to unload, even when time per animal remains unchanged.

The number of stock on a truck was also positively correlated with cleanliness recorded on the morning of slaughter, where higher cleanliness scores indicated dirtier cattle. Higher stocking rates have been shown to increase the risk of dirty cattle (Lowman *et al.* 1997; Gygax *et al.* 2007), which may be a result of more opportunities for dirty individuals to contact other animals and spread contaminants. Additionally, trucks carrying larger numbers of stock (>20) would result in the need for those stock to be separated into smaller wash groups in lairage pens. Consequently, social re-ranking occurs and its effects have been discussed (4.4.2), particularly on stress, which is known to increase defecation (Lauber *et al.* 2006; Kilgour 2012) and thus the likelihood for cattle to become dirtier. However, given the consistency in cleanliness scores recorded across lairage periods (4.4.4.2), the relationship between number of stock on a truck and cattle cleanliness might be more reflective of the space restrictions and increased contacts between animals during the transport journey itself (Davies *et al.* 2000; Small and Buncic 2009).

4.4.4.4 Correlations between observation periods

The vocalisations recorded between unloading, the afternoon of arrival, and the morning of slaughter were not related, suggesting these behaviours occurred as a result of environmental stimuli, rather than a consistently vocal group of animals. However, only one group were observed vocalising during unloading and thus more observations are required to properly understand the relationships with this timepoint.

Whilst cattle cleanliness remained consistent throughout lairage (4.4.4.2), it was not related to lairage wash number or duration. This is surprising as it was anticipated that dirtier cattle would receive more washing than cleaner cattle. A lack in discretion when applying washing suggests mandates may place most emphasis simply on the need to wash, rather than its goal of producing an acceptably clean animal for slaughter. Indeed, the degree to which washing should be carried out and what constitutes a 'clean' animal acceptable for processing is not detailed in the Australian Standard for the hygienic production and transportation of meat and meat products for human consumption AS 4696:2007 (Australia and New Zealand Food Regulation Ministerial Council 2007).

The only strong negative correlation was between ramp (1 = old, 2 = new) and the number of lairage washes, indicating the number of washes applied decreased during use of the new ramp. This may have resulted from differences in staffing and the way they apply washing between the two periods, or due to integration of advice provided from the results of preliminary work to only wash cattle as little as absolutely necessary and no more. Another possibility is that cleaner cattle were consigned during the period of use of the new ramp, requiring less washing. However, unloading ramp was not correlated with cleanliness and as discussed above, cleanliness was not related to lairage washing procedures.

4.5 Conclusion

Lairage is a fundamental component of beef production and the importance of maintaining animal welfare throughout this seemingly small portion of an animal's life is unequivocable. Whilst the factors eliciting a stress response might change between the afternoon and morning lairage periods, animal behaviour suggest stressors are a constant throughout the lairage period.

Chapter 5 | Behavioural responses of beef cattle to pre-slaughter hide washing

5.1 Introduction

Almost all cattle are eventually processed for meat, and an inevitable component of this procedure in commercial settings is time spent in lairage, or abattoir holding yards. Cattle may spend as little as a few hours or remain in lairage overnight. Whilst this forms only a small component of their life, it can have substantial effects on animal behaviour and welfare, meat quality, and the overall value of meat produced.

In Australia, a key component of the period spent in lairage is pre-slaughter hide washing. This practice involves the use of water-based methods such as sprinklers and high-pressure hoses to remove contaminants (mud, faeces, and bedding) from the hide, with the intention to decrease the likelihood of their transfer to the subsequent carcase. This practice falls under the guidelines of the Australian Standard for the Hygienic Transportation of Meat and Meat Products for Human Consumption AS 4696:2007 (Australia and New Zealand Food Regulation Ministerial Council 2007). Whilst readily incorporated as part of normal industry practice throughout Australia, there is a paucity of information on the effectiveness of this procedure at improving hide and carcase cleanliness, and any effects it may have on meat quality, and animal behaviour and welfare.

Animal behaviour is a useful outcome-based tool for assessing animal welfare. Observation of behaviour is a cheap and non-invasive measure compared to other sampling methodologies, which has supported its uptake in many welfare assessments, including behavioural based auditing systems in lairage (Grandin 2010). Performance of certain behaviours can be used to assess the state of an animal’s welfare, and be an indicator of when this is threatened, such as exhibiting fear or avoidance behaviours which can indicate an impact on welfare.

Dark cutting is a term describing beef with a high pH and dark meat colour, resulting from insufficient levels of muscle glycogen at slaughter, the main cause of which is stress (Tarrant 1989). Stimuli activating the stress response and mobilising glycogen during the lairage period may have consequences for dark cutting, as there is insufficient time for glycogen to be replenished prior to slaughter. A previous study indicated a positive relationship between the number of times a slaughter group was washed and the incidence of dark cutting beef within that group (Preston *et al.* 2018). Consequently, identifying and removing stressors from the pre-slaughter environment is of high importance.

The association between pre-slaughter hide washing and meat quality has provided an indication that this practice is initiating the stress response. However, it is not known if washing impacts behaviour in beef cattle, which may also indicate stress. Observing behavioural responses to pre-slaughter washing would assist in understanding the impact of this practice on animal welfare. The aim of this study was to determine if pre-slaughter hide washing affected cattle behaviour in lairage.

5.2 Materials and methods

Data for this observational study were collected from a southern Australian abattoir with a throughput of up to 800 head of cattle per day. Data were collected over two kill days during September and October 2017. Cattle were observed and their behaviour recorded prior-to and during washing on the morning of slaughter. Pasture- and grain-finished cattle from commercial mobs were selected from those scheduled to be processed. The only requirement for inclusion in this study was that cattle would be presented for Meat Standards Australia grading and be certified pasture- or grain-finished. This project was approved by The University of Adelaide Animal Ethics Committee (Approval number S-2016-096).

5.2.1 Animal management

A total of 177 animals from five different vendor consigned mobs were observed during this study (Table 5.1). All cattle arrived at the facilities the day prior to slaughter where they were unloaded and housed in lairage pens overnight. Each lairage pen could accommodate 20 animals. Thus, larger consignments (>20) needed to be separated into smaller groups. No other mixing of mobs occurred. Once mobs were separated into lairage pens, they remained in these groups for processing (wash group). On the morning of slaughter, cattle were washed in an attempt to remove contaminants (mud, faeces, and bedding) from the hide. Slaughter was then conducted by captive bolt gun stun followed by exsanguination.

Table 5.1 Data summary statistics.

	Pasture	Grain	Total
Mobs	4	1	5
Animals	104	73	177
Wash groups	6	3	9

5.2.2 Pre-slaughter hide washing

Washing was conducted using three ordered treatments: lairage washing, high-pressure hosing, and belly washing. Stockmen were responsible for the number and duration of each wash type applied. Cattle could receive more than one wash (water on, water off) of each treatment type, and the length of each wash varied. Lairage washing treatments commenced from 4:30am and groups received their final belly wash approximately 15 minutes prior to slaughter.

Lairage washing was the first form of washing the animals received and the most extensive in number and duration of washes. Lairage washing formed the focus of this study. Cattle were washed a total 3.9 ± 0.5 times totalling 75.9 ± 7.3 minutes of water on. Each lairage pen was fitted with six in-floor sprinklers, used to wash cattle with untreated water. Sprinklers were operated by stockmen from a gantry above the pens, and groups were washed in the order in which they would be presented for slaughter, such that groups scheduled to be slaughtered first would be washed first. Water pressure and temperature were not recorded but the water was not heated.

Following the completion of lairage washing treatments, animals received further washing with a hand-held high-pressure hose, and then in a belly wash pen, but behaviour during these treatments was not recorded as part of this study.

5.2.3 Animal behaviour

Cattle behaviour was observed and recorded prior-to and during lairage washing by scan sampling. Observations of animal behaviour were made from a gantry located above the lairage pens, allowing behaviour of all animals in a group to be seen. The behaviours recorded during this study were shaking, head down, and laying down (Table 3.3). The wash group was also given a group movement score based on the majority of animals in the group (Table 3.4). All behavioural observations were made by the same observer, an animal scientist with beef cattle experience. Cattle behaviour was recorded at seven identified points during the lairage washing process, each comprising a 10 to 15minute observation period.

Cattle behaviour was first observed prior to commencement of any washing treatments (pre-washing) to give an indication of resting behaviour. Each wash group was observed over a 15 minute period; scans were made and the number of animals performing a behaviour was recorded every three minutes.

Cattle behaviour was then observed again once washing commenced. The water would come on in a pen (water on), remain on for approximately 20 minutes, and then stop (water off) until restarted by lairage staff. One combined water on, water off event comprised the first wash (wash 1). Behaviour was recorded at 0, 1, 2, 5 and 10 minutes following the water on and water off event (Figure 5.1). This was then repeated during the second and third wash. The behaviour of wash groups was only recorded during their first three washes.

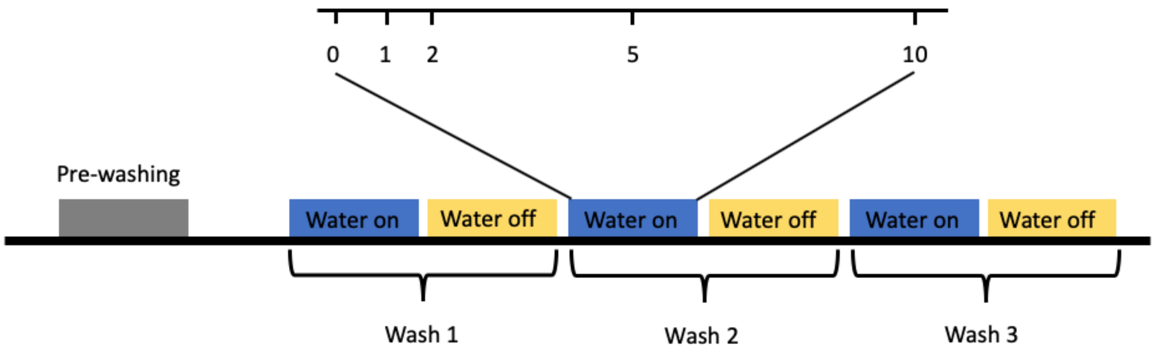


Figure 5.1 Timeline of washing events and points at which behaviour was recorded. Behaviour was first recorded prior to commencement of any washing (pre-washing) using scan sampling every three minutes over a 15 minute period. Behaviour was then recorded following the water on and water off events for the first three washes a wash group received. During these points, behaviour was observed by scan sampling at 0, 1, 2, 5, and 10 minutes following each water on and water off event.

Behavioural observations (shaking, head down, laying) were converted to the percentage of animals in a wash group exhibiting a behaviour for analysis to allow comparison between different sized groups. Behaviours from scans made during pre-washing, and water on or off for a given wash, were averaged for the treatment level (water by wash number). The group movement score is also averaged for the treatment level.

5.2.4 Statistical analysis

Data were analysed using GenStat software (GenStat, 19th Edition, VSN International, Hemel Hempstead, UK) to determine the effect of washing factors on animal behaviour. Data were first summarised (mean, median, range, standard deviation, coefficient of variation) and checked for normality. One was added to each group movement score and $\log_{10}$ transformed prior to analysis to remove skewness. Analysis from these data were then back transformed and results are presented here log-normal. An approximate standard error is reported for this trait as data have been back transformed.

Generalised linear models were developed to determine the effect of washing treatment on the behaviour of cattle in a wash group. Behaviours were analysed separately (head down, shaking, laying down, group movement). All models included fixed effects of feed type (pasture, grain) water (pre-wash, on, off), and wash number (pre-wash, 1, 2, 3). Each factor was first tested individually before fitting two- and three-way interactions. The same maximum model using all three factors and their interactions was fitted to each behaviour for consistency. Significance was defined as $P<0.05$. The effects of variables on behaviour are reported as the predicted mean for the proportion of animals in a wash group exhibiting a behaviour (head down, shaking, laying down), or the effect on group movement score (0-3). Predicted means from the maximum model were averaged to determine means for lower order interactions. Results are presented as mean ± standard error unless stated otherwise.

5.3 Results

The most common behaviour recorded during this study was head down, followed by shaking, and a limited number of animals were observed laying down (Table 5.2). An average score of 0.3 was recorded for group movement. Behaviours were highly variable as indicated by coefficient of variation (Table 5.2). All washing factors fitted in the final models had significant effects on behaviour individually and in some interactions (Table 5.3).

Table 5.2 Summary statistics (mean, median, range, standard error of mean, standard deviation, coefficient of variation) of behaviours observed throughout this study, presented as percentage of the group exhibiting the behaviour (%), and group movement score. Behaviours are pooled for all observation points (prior to- and during-washing).

Behaviour	Shaking	Head down	Laying down	Group movement
Mean (median)	7.2 (5.6)	23.3 (19.3)	0.2 (0)	0.3 (0)
Range	0 – 25	0 - 80	0 - 5	0 - 2
Standard deviation	5.9	16.7	0.7	0.5
Coefficient of variation (%)	83	72	401	156

Table 5.3 Tests of significance for the effects of feed type, water, and wash number and their interactions on shaking, head down, laying down, and group movement.

Fixed term	Shaking	Head down	Laying down	Group Movement
Feed type	<0.001***	0.02*	0.004**	0.758
Water	<0.001***	<0.001***	<0.001***	0.479
Wash number	<0.001***	0.017*	0.077	<0.001***
Feed type x Water	0.216	0.555	<0.001***	0.628
Feed type x Wash number	0.841	0.002**	0.241	0.422
Water x Wash number	<0.001***	0.697	0.616	0.576
Feed type x Water x Wash number	0.560	0.115	0.168	0.148

*, **, *** highlight significance at $P < 0.05$, 0.01, and 0.001, respectively.

5.3.1 Shaking

Shaking behaviour was affected by feed type, water, and wash number, as well as the interaction between water and wash number ($P<0.001$; Figure 5.2). More pasture-finished cattle (7.6%) were observed to be shaking than grain-finished cattle (3.9%). Across feed types, a mean 1.3% of cattle were observed shaking during the pre-wash period, however following washing events this increased significantly. During water on, shaking increased to 7.1%, and remained elevated in comparison to the pre-wash period during water off (5.6%). The highest proportion of shaking was recorded during wash 1 at 8.4% before dropping over subsequent washes to a level of 4.9% during wash 3.

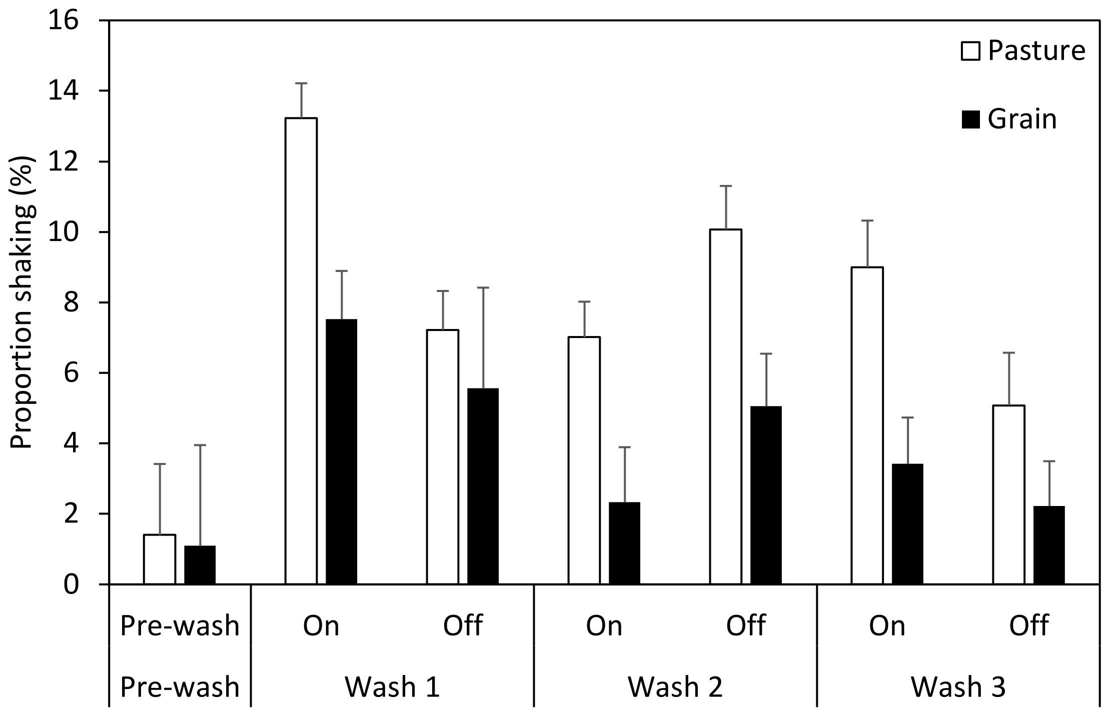


Figure 5.2 The predicted mean ± standard error of the proportion of a group scored shaking for each (treatment level) water by wash number interaction. Pasture and grain finished groups are separated.

5.3.2 Head down

Head down behaviour responded to all washing factors individually, as well as the interaction between feed type and wash number (feed type P=0.02; water P<0.001; wash number P=0.017; feed type x wash number P=0.002; Figure 5.3). Head down was recorded more in pasture- (21.8%) than grain-finished cattle (17.4%), and when water was on (29.8%), than during water off (13.3%) or in the pre-wash period (7.9%). The lowest observations of head down for pasture- and grain-finished cattle were during the pre-wash period, 10.0% and 5.7% respectively. In pasture finished cattle, head down increased to 19.5% during the first wash before reaching a peak of 30.9% in the second wash, and returned to 20.8% during the final wash. The highest level of head down in grain finished cattle was recorded during the first wash at 22.6%, before lowering to 16.6% and 18.9% in the second and third washes, respectively.

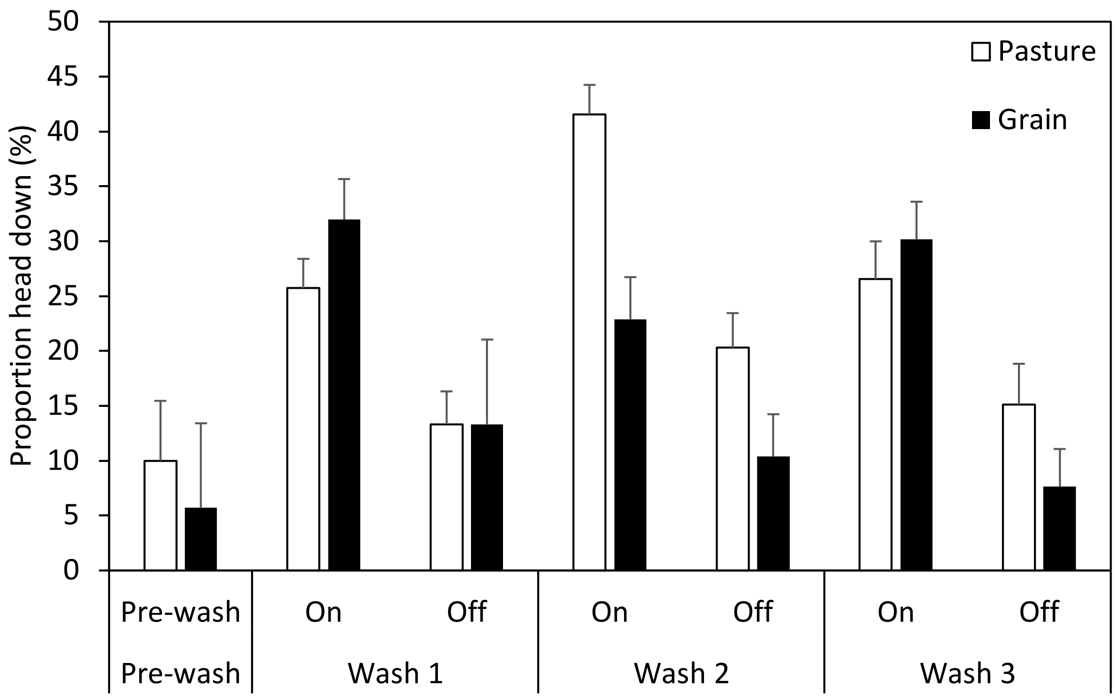


Figure 5.3 The predicted mean ± standard error of the proportion of a group scored head down for each (treatment level) water by wash number interaction. Pasture and grain finished groups are separated.

5.3.3 Laying down

Feed type, water, and their interaction affected laying down behaviour (P=0.004, <0.001, <0.001; Figure 5.4). Grain-finished cattle were observed laying down more than pasture-finished cattle, and this behaviour significantly decreased once washing commenced. During the pre-wash period, 6.9% of grain- were observed laying down compared to only 2.8% of pasture-finished cattle, with only 0.0 – 0.3% of cattle observed laying down during water on and off for both feed types.

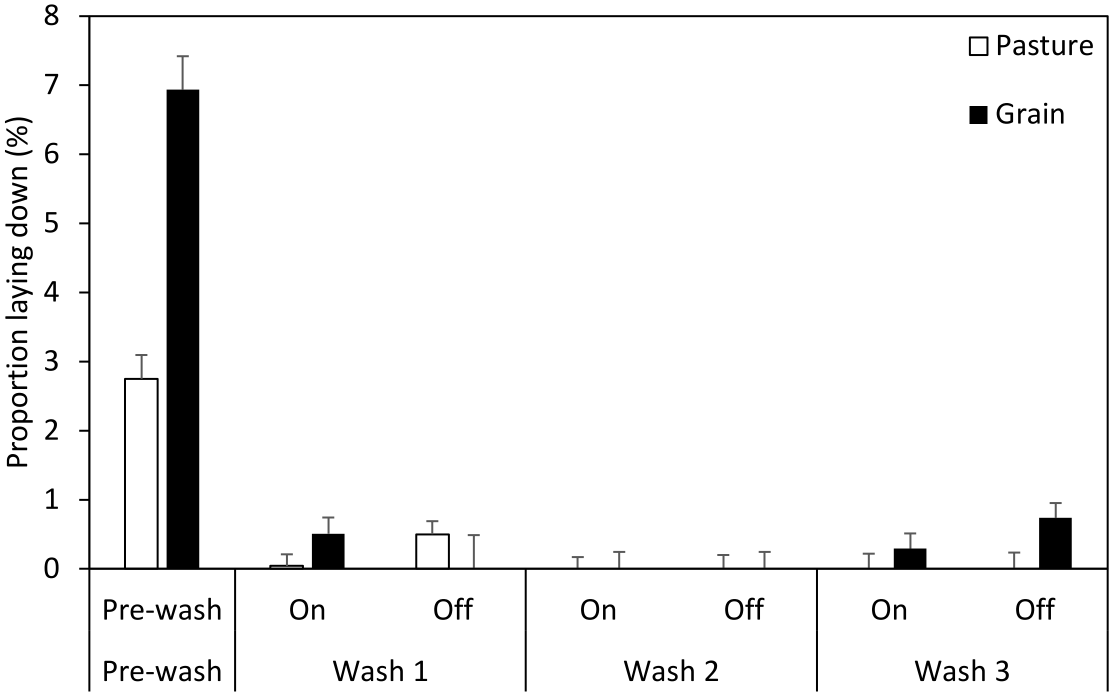


Figure 5.4 The predicted mean ± standard error of the proportion of a group scored laying down for each (treatment level) water by wash number interaction. Pasture and grain finished groups are separated.

5.3.4 Group movement

Wash number significantly affected group movement (P<0.001; Figure 5.5). Group movement score was 1.4 and 1.3 during pre-wash and wash 1, respectively. This then fell to a low of 1.1 during wash 2 before returning to 1.4 during the final wash.

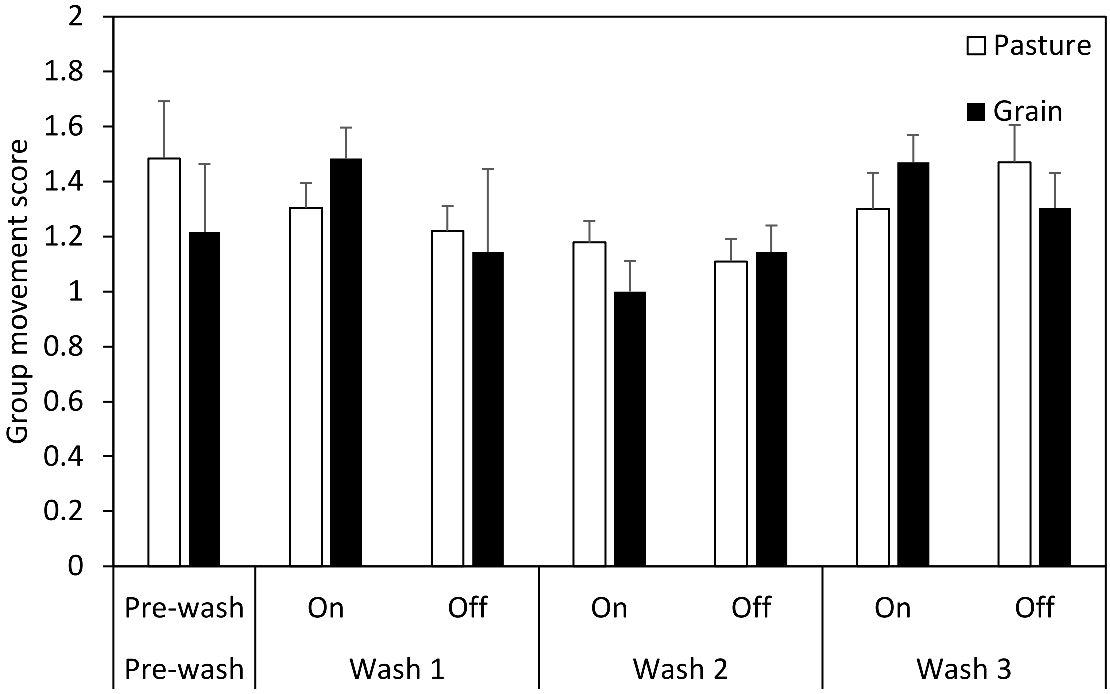


Figure 5.5 The predicted mean ± approximate standard error of the group movement score for each (treatment level) water by wash number interaction. Pasture and grain finished groups are separated.

5.4 Discussion

To our knowledge, there are currently no published studies reporting the effect of pre-slaughter hide washing in lairage on the behaviour of beef cattle. This study indicates pre-slaughter hide washing with in-floor sprinklers results in a change in shaking, head down, and laying behaviours, as well as group movement, when compared with resting levels for each behaviour recorded prior to washing. The behaviours recorded here were highly variable, however analysis indicated they were affected by feed type, water, and wash number, and interactions between these factors.

Shaking and head down increased significantly following the commencement of washing during water on, whilst laying down significantly decreased. Following water off, these behaviours did not return to the resting level observed prior to washing; shaking and head down remained elevated and laying down lowered following washing. These results showing an increase in avoidance behaviour (head down, shaking) and a decrease in resting behaviour (laying down) in response to washing are similar to those reported by others, as discussed below.

The use of potable water sprinklers to mitigate heat stress in dairy cattle has been reported to elicit behavioural responses of cows on exposure to water similar to those in the study herein. Cattle spent more time with their head down when exposed to sprinklers (Kendall *et al.* 2007; Chen *et al.* 2016a) and were five times more likely to have their head lowered on exposure to rain and wind than when sheltered (Tucker *et al.* 2007). Cattle also show a preference for shade over sprinklers (Schütz *et al.* 2010). Furthermore, cattle have been observed to stand with their heads under a feed bunk such that they are covered when sprinklers are on (Tucker *et al.* 2007). This increase in head down when under sprinklers observed in all of these studies is likely a form of avoidance behaviour to reduce head exposure to water. Head down behaviour has also been linked to an increase in plasma cortisol concentration (Hemsworth *et al.* 2011). Conversely, cattle can show a preference for and spend more time in areas with sprinklers as ambient temperature rises in summer conditions and will both stand and lay during exposure (Legrand *et al.* 2011; Parola *et al.* 2012; Chen *et al.* 2016b). Head position during water exposure was not reported during these studies.

In addition to immediate changes in behaviour, the use of water sprinklers on dairy cattle can also result in longer-term physiological changes. Skin and body temperature decreases along with respiration rate when sprinklers are used (Kendall *et al.* 2007; Chen *et al.* 2015; Tresoldi *et al.* 2018). Cooling treatments using sprinklers have not been shown to immediately affect circulating insulin, plasma glucose, and nonesterified fatty acid, but can cause changes over a longer period (Tao *et al.* 2012). This potential for a decrease in body temperature due to water exposure may be aversive during colder environmental conditions, including when cattle are washed in lairage year-round, and thus increase the amount of energy expended on thermoregulation. A thermoregulatory response would mobilise energy stores pre-slaughter which could be a contributing factor to the increase in dark cutting incidence associated with pre-slaughter washing (Chapter 3). Increased plasma cortisol concentration can also occur by preventing cattle from laying down (Fisher *et al.* 2002), as observed post-washing, and thus may also lead to mobilisation of energy stores resulting in dark cutting.

The decrease in laying down observed following pre-slaughter hide washing may further contribute to thermoregulatory and physiological changes. Studies elsewhere have also reported cattle spend less time laying down when kept outside and exposed to overhead sprinklers (Tucker *et al.* 2007). The ambient temperature at which cattle increase body heat production is lower when animals are laying down rather than standing (13.5ºC while lying, 17ºC standing) (Schrama *et al.* 1993), thus cattle spend more time laying down to maintain body temperature on exposure to cold and/or wet conditions (Malechek and Smith 1976; Gonyou *et al.* 1979; Redbo *et al.* 2001). However, the decrease of laying and increase in head down observed in this study following washing suggest the motivation for cattle to avoid water is greater than that to make a postural change and maintain body temperature, which may be lowered by washing. Furthermore, cattle may lay down less following washing due to the wet floor that results. Studies suggest moisture content plays a part in laying, and cattle prefer dry surfaces for this behaviour (Keys *et al.* 1976). Thus, the lack of cattle observed laying following the commencement of washing may partially be a result of the subsequent increase in moisture content on the pen floor. One factor that may also contribute to laying down decreasing following washing is the diurnal pattern of this behaviour. Cattle spend more time laying overnight than during the day, and the nature of this study meant pre-washing behaviours were always recorded prior to washing, which occurred as the morning progressed.

Shaking behaviour also increased following the commencement of pre-slaughter hide washing. Increases in observations of shaking in cattle have also been made following other aversive procedures such as dehorning (Park *et al.* 2017) and liver biopsy (Mølgaard *et al.* 2012). Whilst it could be anthropomorphised that washing is not as aversive a procedure as dehorning or biopsy, the same behavioural responses are reported, suggesting cattle perceive them similarly. The increase in shaking observed in this study is likely a result of discomfort resulting from washing, and may aid in reducing the amount of water on the head. Shaking behaviour decreased slightly during the second and third wash simultaneously with an increase in head down, suggesting avoidance by lowering of the head reduced exposure to water, and thus the need to shake from discomfort.

Group movement did not follow a clear trend over the duration of washing. Cattle will perform avoidance behaviours like moving away from stimuli when in fear (Probst *et al.* 2012) and movement during washing may have been to escape this procedure. Group movement was lowest when head down behaviour peaked for pasture finished cattle during the second wash, suggesting attempts to escape washing were replaced with this avoidance behaviour to minimise head exposure to water. Both movement and head down behaviours occur in response to aversive stimuli, highlighting the continued attempt to remove the stressor.

5.5 Conclusion

This study shows the behaviour of beef cattle changes in response to pre-slaughter hide washing in lairage. Following washing, behaviours remain changed and do not return to the resting level observed prior to the commencement of washing. Avoidance behaviours indicative of stress like shaking and head down increase, and the resting behaviour laying down decreases following washing. The results of this study suggest pre-slaughter hide washing is a stressful event for beef cattle which could negatively affect welfare. Furthermore, literature elsewhere has reported links between behaviours observed here and physiological changes, which may impact meat quality factors like dark cutting. The continued use of pre-slaughter hide washing to improve carcase hygiene requires refinement to ensure the methods used are minimally aversive, reducing their impact on animal behaviour and welfare.

Chapter 6 | Factors affecting beef carcase hygiene

6.1 Introduction

A key factor underpinning Australia's strong international trade market is the superior hygiene of beef carcases, which have contamination levels some 90-99% lower compared to those of other countries (Sumner *et al.* 2018). Carcase hygiene is routinely monitored to minimise the risk of microbial foodborne disease originating from meat products and to substantiate food safety claims. In Australia, the National Carcase Microbiological Monitoring Program (NCMMP) was implemented, originally in 1998 as the *E. coli* and *Salmonella* Monitoring Program, to monitor the hygiene performance of all export registered establishments in compliance with export legislation (Department of Agriculture Water and the Environment 2020b). Beef carcases are monitored for Standard Plate Counts (SPC), *Escherichia coli*, coliforms, and *Salmonella spp*. at a frequency relative to production volume of the plant (Department of Agriculture Water and the Environment 2020b). Standard Plate Counts provide an indication of environmental hygiene of the meat production process, whilst *E. coli* and coliforms assess enteric contamination originating from the animal (Ghafir *et al.* 2008).

Whilst the flesh of healthy animals is inherently sterile (Bacon 2005; Aiyegoro 2014), contamination can occur exogenously at any time from the point of sticking until consumption. The animal hide and gastrointestinal tract are recognised as the major sources of contamination to the carcase (Ayres 1955; Grau and Smith 1974). Farmed ruminants are asymptomatic carriers of human enteric pathogens which are prolific in the rumen and gastrointestinal tract.

These pathogens are shed in the faeces (Hutchison *et al.* 2005) and result in contamination of the hide, which can then be transferred to the carcase during hide removal and evisceration (McEvoy *et al.* 2000). The potential for contamination directly from the gastrointestinal tract or its contents is minimal, providing the gastrointestinal tract is not ruptured during evisceration (Nottingham *et al.* 1974; Grau 1979).

Many studies focus on a particular factor and its relationship with microbial prevalence in just one processing environment, which is likely the cause of the differences in results between studies. The prevalence of some specific Shiga toxin-producing *E. coli* strains differs between plants (Cernicchiaro *et al.* 2020) and variation in overall hygiene between processors in Australia is evident (Kiermeier *et al.* 2006). A large study investigating these differences identified that no one factor was able to explain the variation between plants, and that the combination of factors related to the incoming stock and processing practices was most important (Kiermeier *et al.* 2006). Thus, it is imperative to consider factors affecting carcase hygiene for each processor individually given the importance of the combination of processing practices on hygiene.

Whilst Australian beef carcases have 1-2 $\log_{10}$ less contaminants than those produced in other countries (Sumner *et al.* 2018), maintaining and improving hygiene remains of great importance to prevent carcase contamination with food borne pathogens. In investigating methods for hygiene improvement, it is key to understand factors related to increased microbial contamination and manage these accordingly when planning investigations. The aim of this study was to understand animal and environmental factors related to beef carcase hygiene to guide future sampling at a processor using site specific historical data.

6.2 Materials and methods

This study was conducted using historical data collected from a large southern Australian beef processor. A total of 2,929 samples recorded as part of the NCMMP were analysed in this study (Table 6.1). A carcase sample could be analysed for either SPC, *E. coli* and coliform, or *Salmonella*, or a combination of these. The number of samples collected each year fluctuated proportionally to the total number of carcases processed. Salmonella results from 2016 were not available. Samples analysed in 2020 were limited to those collected between 1st January and 29th May.

Table 6.1 Summary of sample type and year of collection from the National Carcase Microbiology Monitoring Program data analysed in this study. The testing performed on a sample from a carcase varied, with the possibility of testing carcases for one or multiple of Standard Plate Count (SPC), E. coli and Salmonella.

	SPC	*E. coli*	*Salmonella*	Total
2016	394	394	*	482
2017	401	402	480	485
2018	637	639	128	786
2019	695	693	137	854
2020	258	259	51	322
Total	2,385	2,387	796	2,929

* Salmonella results for 2016 were not available.

6.2.1 National Carcase Microbiology Monitoring Program data

Data for this study were collected and recorded under the NCMMP and provided for analysis by the processor. Carcases were sampled randomly at a regulated frequency based on production volume at the processing facility. Samples were collected at a rate of 1 per 300 carcases processed for both SPC and *E. coli*, and 1 per 1,500 carcases processed for *Salmonella* (Department of Agriculture Water and the Environment 2020b). Carcases to be sampled were selected using a random generator, which included the side (left or right) of the carcase to be sampled. Samples were collected by experienced staff at the facility and refrigerated at 4°C until transported for processing at a department approved laboratory. Samples were packed for transport in insulated containers with cold packs to maintain temperature at 0-5°C and sent using same day/overnight delivery. Samples were typically collected in the morning (5-9am) the day after slaughter following at least 12 hours of active carcase chilling. All samples were sent for processing on the same day they were collected. Due to transport restrictions, carcases required to be sampled on a Friday were held over until the following Monday, at which point a sample would then be collected and transported to maintain sample integrity.

6.2.2 Carcase sampling

Sampling was conducted following guidelines set out by the NCMMP (Department of Agriculture Water and the Environment 2020b). Briefly, a sterile sponge moistened with 10mL buffered peptone water was used to collect samples from specified carcase sites on the flank, brisket, and inside butt (Figure 6.1). Technicians collecting the samples wore sterile gloves changed between each carcase, to hold the sponge and collect samples. A $10cm^2$ template provided a boundary for sampling using 10 horizontal and 10 vertical strokes with the sponge on the carcase at each location. The flank and brisket were sampled first using the same side of the sponge. The opposite side of the sponge was then used to sample the butt. The sponge was then returned to a labelled Whirl-Pack® (Nasco sampling, Madison, WI) for storage and the remaining 15mL buffered peptone water was added (total = 25mL) before transport and analysis. Both sides (left and right) of all sampled carcases were retained until laboratory results were received.

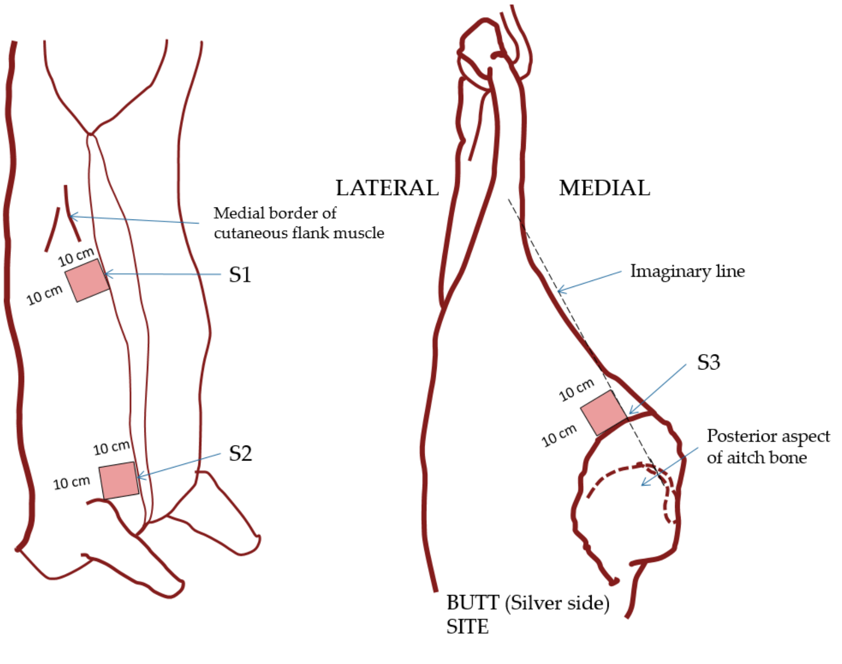


Figure 6.1 Illustration of the three carcase sites sampled as part of the National Carcase Microbiology Monitoring Program. Samples are collected using a sponge wiped over a 10cm^2 area at three carcase sampling sites. (Department of Agriculture Water and the Environment 2020b).

6.2.3 Sample analysis

All samples were analysed at a laboratory approved by the Department of Agriculture and Water Resources, which required accreditation by the National Association of Testing Authorities (ISO/IEC 17025). Samples were analysed using protocols listed under the Approved Methods for Microbiological Testing of Meat and Meat Products (Department of Agriculture Water and the Environment 2020a) as follows.

6.2.3.1 *Standard Plate Count*

Standard plate count (SPC) was determined using Aerobic Plate Count in Foods (Petrifilm™ Method) – AOAC 990.12. Briefly, samples were diluted 1:10 with buffered peptone water and 1mL of the serial dilution was plated onto SPC 3M™ Petrifilm™ (3M, Minnesota, USA). Plates were then incubated at 35°C for 48 hours, following which all red coloured colonies were counted and the result expressed in colony forming units per square centimetre (CFU/cm^2).

6.2.3.2 *E.coli and coliforms*

E. coli and coliforms were assessed by *E. coli* Petrifilm™ – AOAC 991.14. Briefly, samples were enumerated without further dilution by plating 1mL onto 3M™ Petrifilm™ (modified Violet Red Bile media, Minnesota, USA) and then incubated at 35°C for 48 hours. All blue colonies associated with gas were counted as *E. coli*, and red colonies with gas as coliforms. The coliform count provided was the sum of the red and blue colonies. *E. coli* and coliform result were expressed in colony forming units per square centimetre (CFU/cm^2).

6.2.3.3 *Salmonella*

Determination of Salmonella was by VIDAS®UP Salmonella method (VIDAS® SPT) AOAC 071101. Briefly, additional buffered peptone water was added to the sample, bringing the volume total to 60-100mL, before incubation at 42°C for 18-25 hours. A portion of boiled post enrichment broth was used to perform the VIDAS® SPT test in the VIDAS® instrument (bioMérieux, Askin, Sweden). Any Salmonella positive samples were confirmed with enrichment broth following AS 5013.10.

6.2.4 Sample results

Following sample analysis at a laboratory, results were provided to the abattoir. Analysis of SPC and *E. coli* were reported as colony forming units per square centimetre (CFU/cm^2), and *Salmonella* as detected or not detected. Results were assessed for performance against standards defined by the NCMMP (Department of Agriculture Water and the Environment 2020b). For SPC and *E. coli*, values falling at or below a limit, m, are acceptable, and any exceeding a limit, M, are unacceptable (Table 6.2). Thus, each carcase result was then classified as acceptable (<m), marginal (>m but <M), or unacceptable (>M). Carcases returning a marginal result triggered a monitoring window comprising 15 (n) consecutive samples. A window would fail if the number of marginal results exceeded three (c), or if a single result exceeded M, which would also trigger an alert. Carcases returning an acceptable or marginal result were returned to processing and carcases with an unacceptable result were condemned. For *Salmonella*, a positive result triggered a window for steers and heifers of 82 samples, or for cows and bulls 58 samples, with a set limit of one or two permitted positive samples, respectively. All carcase sample results were recorded and stored in a system maintained by the abattoir. The factors recorded along with the laboratory analysis result for each sample and used for this analysis are presented in Table 6.3.

Table 6.2 Performance requirements for Standard Plate Count and E. coli sampling as set out by the National Carcase Microbiology Monitoring Program (Department of Agriculture Water and the Environment 2020b). Sample analysis indicates an acceptable (<m), marginal (>m but <M), or unacceptable (>M) result for steers, heifers, cows, and bulls based on the levels defined for m and M. A marginal result triggers a window of n consecutive samples, in which c marginal results are permitted. A single result exceeding M will result in a fail and trigger an alert.

	Window (n)	Marginal results (c)	m CFU/cm^2	M CFU/cm^2
Standard Plate Count	15	3	1,000	31,625
E. coli	15	3	0	20

Table 6.3 The information recorded and stored for each carcase sampled under the National Carcase Microbiology Monitoring Program, in addition to the result of laboratory analysis.

Factor	Description
Year	The year the sample was collected
Month	The month the sample was collected
Purchase order	A reference number reflecting a batch of samples sent together for laboratory processing
Send date	The date (dd/mm/yy) a sample was sent on transport to the processing laboratory
Sample date	The date (dd/mm/yy) a sample was collected
Sample time	The time (hh:mm) a sample was collected
Kill date	The date the animal was slaughtered
Category	The sex (cow or steer) of the animal
Shift	The shift (day or night) the sample was collected
Body number	A number indicating the order an animal was slaughtered, where 1 indicates the first animal on any given day
Side	The side of the carcase (left or right) being sampled
AUS-Meat category	The type of carcase being sampled, grouped by dentition and sex as defined by AUS-Meat (AUS-MEAT 2018). Categories included: beef (A), bull (B), cow (C), prime beef (PR), prime steer (PRS), steer (SS), yearling beef (Y), young beef (YG), young steer (YGS), young prime beef (YP), young prime steer (YPS), yearling steer (YS)
Feed type	The feed type of the animal (grass or grain)
Chiller	The chiller (0-9) in which the carcase was stored

6.2.5 Statistical analysis

Data were analysed using R version 3.6.3 (R Core Team 2020) with the packages ASReml-R (Butler *et al.* 2009) and ggplot2 (Wickham 2016). Data were first summarised and checked for normality. During the sampling period *E.coli* and coliform distributions had negligible variation, and *Salmonella* were not detected; thus analysis focused on SPC. Values falling below the limit of detection (<0.083 CFU/cm^2) for SPC testing were assigned a value of 0.083 CFU/cm^2 for analysis. SPC values did not follow a normal distribution (skewness = 19.4, kurtosis = 483) and were log_{10} transformed prior to analysis which normalised skewness and kurtosis (skewness = 0.2, kurtosis = 3). Back transformation was not performed and all results are reported as log_{10} CFU/cm^2 values, unless otherwise indicated.

Factors recorded as part of the NCMMP (Table 6.3) were analysed using linear mixed models to evaluate their effect on SPC. Purchase order was fit as a random term for all models. Factors were first tested individually in univariate models, from which significant terms were further tested using first and second order interactions. All significant factors and their interactions were then fit in a final model. Factors were removed in order of least significance, starting with higher order interactions, until only significant factors or non-significant factors forming part of a higher order interaction remained. Significance was defined as a P value less than 0.05 using incremental Wald tests.

Some fixed terms from the final model were further explored by fitting as random components to understand the variance associated with each factor. An autoregressive type 1 time series analysis was also conducted using Microsoft Excel (Microsoft Corporation, 2018) to explore the relationship between contiguous days, weeks, months, and years sampled. The correlation/R^2 value was calculated.

6.3 Results

E. coli and coliform had little variation with 99.4% and 97.2% of the samples analysed falling below the limit of detection, respectively (Table 6.4). For *E. coli* the number of marginal and unacceptable results throughout the study period were ten and two, and for coliform, forty and six, were marginal and unacceptable, respectively. Thus, to avoid effects resulting from points of high leverage, only SPC values were considered for analysis. SPC results ranged from <0.83 CFU/cm2 to 82,000 CFU/cm2, with 20.1% of values falling below the limit of detection for sampling of <0.83 CFU/cm2.

Table 6.4 Distribution of results for Standard Plate Count (SPC), E. coli and Coliform testing, reported in CFU/cm^2. The number of samples falling below the limit of detection (LOD) for analysis is also indicated.

	SPC ($\log_{10}$)	*E. coli*	*Coliform*
Minimum	<0.83 (< -0.08)	<0.083	<0.083
1st Quartile	0.83 (-0.08)	<0.083	<0.083
Median	5.00 (0.7)	<0.083	<0.083
Mean	268.3 (2.4)	0.3391	0.468
3rd Quartile	20.0 (1.3)	<0.083	<0.083
Maximum	82,000 (4.9)	660	560
n <LOD (%)	480 (20.1)	2,372 (99.4)	2,322 (97.2)

Year, month, feed type and chiller significantly affected SPC when analysed initially in univariate models (P<0.005). In the multivariate linear mixed effects model, year, month and their interaction remained highly significant and had the highest F-values (P<0.001, Table

6.5); the interaction between year and month also had the largest variance component of those tested. Chiller was another highly significant factor with a large F-value ($P<0.001$, Table 6.5). The feed type by month interaction was also significant ($P=0.016$, Table 6.5).

Table 6.5 The final linear mixed model developed from backward elimination to determine the effect of factors on $\log_{10}$ standard plate count. Purchase order was fit as a random term. Fixed factors were fit in the order they appear in the table. The variance component associated with some fixed factors was also determined.

Factor	P-value	F-value	Variance (%)
Year	0.00000	26.82	
Month	0.00000	5.51	
Chiller	0.00002	4.21	
Feed type	0.43395	0.61	
Year x Month	0.00000	6.61	11.4
Month x Feed type	0.01576	2.13	0.1

6.3.1 Factors affecting Standard Plate Count

The predicted SPC $\log_{10}$ of the years tested were significantly different to each other and values increased across years (Figure 6.2 A). There was a variation of approximately 1.5 $\log_{10}$ CFU/cm^2 between the beginning and end of the sampling period in 2016 and 2020, respectively. The greatest difference in predicted SPC $\log_{10}$ between months were March and December, which differed by approximately 0.8 $\log_{10}$ CFU/cm^2 (Figure 6.2 B). Contiguous months tended to be similar and there was no significant difference between the months of January, February, April, May, June, July, October and November. The predicted SPC of grass finished cattle was approximately 0.15 $\log_{10}$ CFU/cm^2 higher than grain finished cattle (Figure 6.2 C). Chillers differed in predicted SPC by up to 0.4 $\log_{10}$ CFU/cm^2 (Figure 6.2 D). Chillers one, two, three and five had the highest predicted SPC and were significantly different to all other chillers, but not each other. Additionally, chiller nine had the lowest prediction and differed significantly to chillers four and eight, but none of these three chillers were different to six and seven. No predicted values were greater than m, the level set for acceptable results (Department of Agriculture Water and the Environment 2020b), equivalent to 3.0 $\log_{10}$ CFU/cm^2.

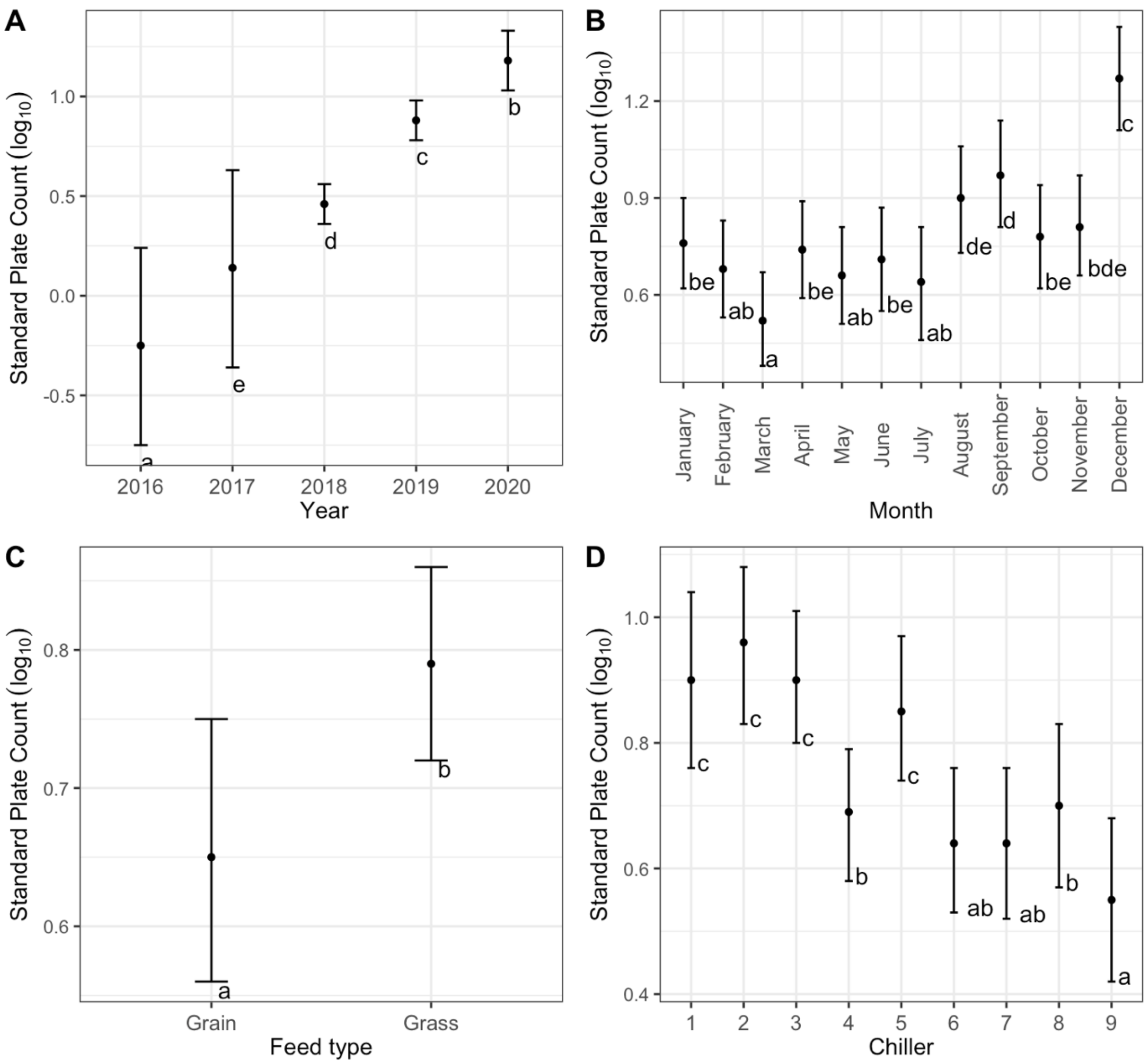


Figure 6.2 The predicted Standard Plate Count ± standard error ($\log_{10}$ CFU/cm^2) for year (A), month (B), feed type (C) and chiller (D). Letters indicate groups which differ significantly within each graph.

The predicted SPC $\log_{10}$ for both grass and grain feed types were at their lowest in March and peaked in December (Figure 6.3). November to January were higher for grain finished cattle compared to other months. However, grain finished cattle had a lower SPC than grass finished cattle during February, March, April, July and October. A greater range in values were predicted for grain finished than grass finished cattle, which ranged from 0.25-1.5 $\log_{10}$ CFU/cm^2 and 0.45-0.95 $\log_{10}$ CFU/cm^2, respectively.

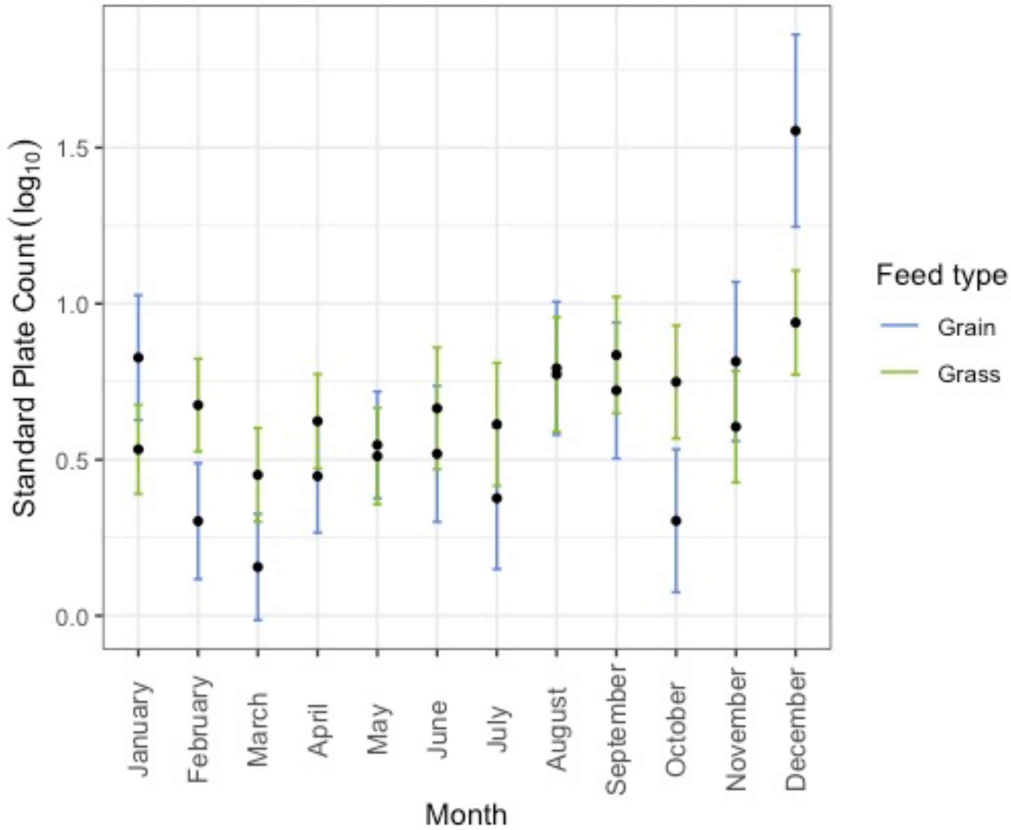


Figure 6.3 Predicted Standard Plate Count ± standard error ($\log_{10}$ CFU/cm^2) by month for both grain and grass feed types.

The interaction between month and year showed great variation in predicted SPC $\log_{10}$ CFU/cm^2 within months (Figure 6.4). A difference of up to more than 1.5 $\log_{10}$ CFU/cm^2 was observed in August over years. March 2020 had a predicted SPC more than 1.0 $\log_{10}$ CFU/cm^2 higher than March of any other year. Within years, an increase was observed in August and

September when compared to July and October, apart for 2018, where there was a decrease from July to August. There was a decrease in predicted SPC from August to October, excepting 2017 with an increase predicted in September. This was followed immediately by an increase from October through to December, except for 2019, which decreased between October and November and remained similar in December. Additionally, monthly predictions for all months in 2020 were higher than for the same months in 2016 and 2017. The greatest difference within a year was 2017, where months varied by as much as 2.0 $\log_{10}$ CFU/cm^2.

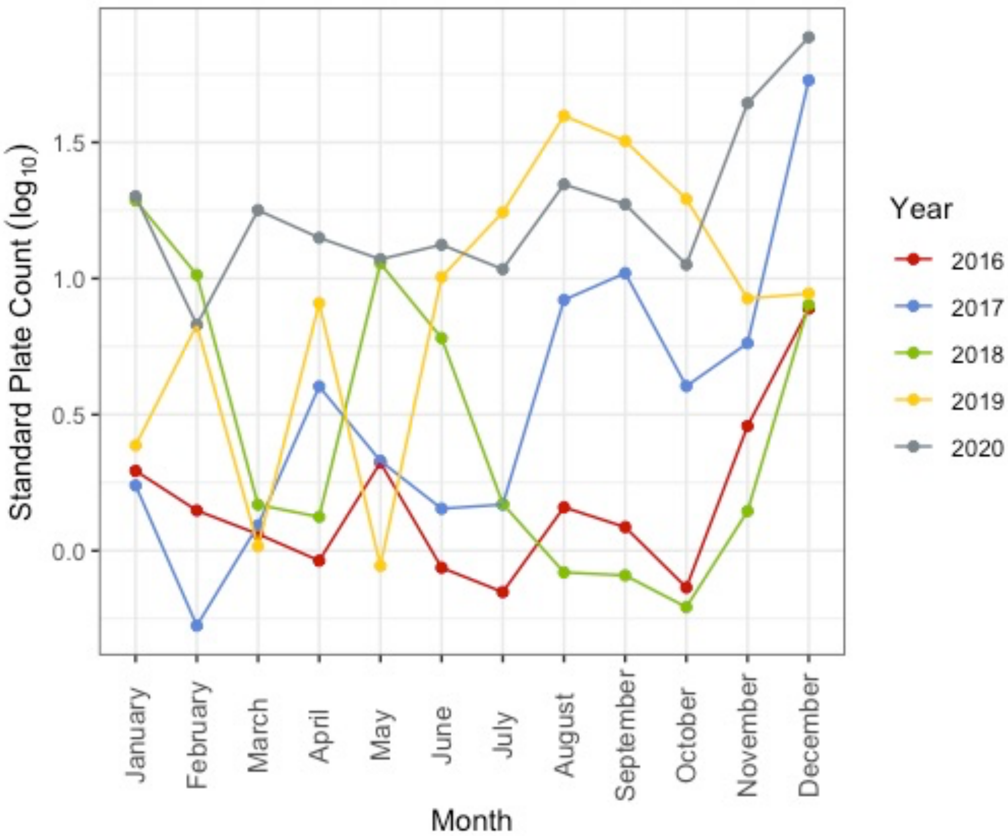


Figure 6.4 Predicted Standard Plate Count ($\log_{10}$ CFU/cm^2) by month for each year sampled.

6.3.2 Time series analysis

An autoregressive type 1 analysis indicated no relationship between contiguous days and weeks sampled (Table 6.6). However, there was a moderate relationship between contiguous months and years. Results also showed a trend where the relationship between time periods increased as the time period lengthened from days to years.

Table 6.6 The r and R^2 value from autoregressive type 1 analysis of contiguous time periods.

	R^2	r
Day	0.0653	0.2555
Week	0.2629	0.5127
Month	0.4222	0.6498
Year	0.6289	0.7390

6.4 Discussion

Many factors impact the microbial status of beef carcases and understanding their effects on contamination is important to mitigate risks to food safety. This study identified factors associated with microbiological contamination of beef carcases, including year, month, feed type and chiller. A relationship in microbial contamination level between sampling periods was also reported. Although this study indicated factors linked to increased microbial contamination, the overall carcase hygiene remained well within acceptable limits reflecting the superior hygiene status of Australian beef.

6.4.1 Overall carcase hygiene

Standard plate counts in this study had a mean and median of 2.43 $\log_{10}$ CFU/cm^2 and 0.70 $\log_{10}$ CFU/cm^2, respectively. These values are higher than those recorded nationally over the previous ten years where the mean total viable count cycled around 1.0 $\log_{10}$ CFU/cm^2

(Sumner *et al.* 2018), although most predictions in this study did fall below this level. The mean *E. coli* was -0.47 $\log_{10}$ CFU/cm^2 while the median was below the limit of detection of -1.08 $\log_{10}$ CFU/cm^2, both well under the national *E.coli* mean of 3.0 $\log_{10}$ CFU/cm^2 for beef (Sumner *et al.* 2018). The prevalence of *E. coli* and *Salmonella* in this study was 0.5% and 0%, falling below the national prevalence of 4% and 0.5%, respectively (Sumner *et al.* 2018).

This study recorded higher SPC and lower *E. coli* values compared to national averages, suggesting most contamination resulted from the processing environment, rather than originating from the animal (Ghafir *et al.* 2008). A high proportion of grass finished cattle (76.7%) were processed and sampled in this study which likely contributed to the low enteric contamination, given grain finished cattle typically have higher concentrations of *E. coli* than grass finished cattle (Callaway *et al.* 2003). Furthermore, Australian cattle are recognised as having relatively clean hides, attributed to the high proportion of grass finished cattle processed in Australia (Sumner *et al.* 2018). This is reflected in the feed type of animals in this study, contributing to a reduction in potential enteric contamination from the hide (Ayres 1955; Grau and Smith 1974). The high mean SPC values could also be a result of the lower number of samples analysed in this study compared to the national database, thus higher values are having a greater effect on the mean.

Although the variation in carcase microbiology has been explored with consideration to contamination of both the incoming animal and subsequent process controls, no one factor is a reliable predictor for carcase hygiene in Australian processing (Kiermeier *et al.* 2006). The most effective control for decreasing microbial contamination results from multiple sequential prevention and intervention measures (Bacon *et al.* 2000; Barco *et al.* 2015). Factors which should be explored in future work that when combined could contribute to the lower-than-average *E. coli* and *Salmonella* recorded at this plant include the feed type of incoming cattle, the distance travelled to slaughter, and animal cleanliness on arrival, along with slaughter floor processes such as chain speed and hide removal method.

6.4.2 Factors affecting carcase hygiene

Year, month, and year by month had a highly significant ($P<0.001$) effect on SPC, with the interaction attributed to 11.4% of the random variance. There was also a moderate relationship between SPC levels in contiguous months and years. The month by year interaction indicates some months in some years provide particular problems for SPC which may be due to seasonal differences, further to the trends observed for year and month individually. The greatest difference within a year was recorded in 2017, where the range in SPC was 2.0 $\log_{10}$ CFU/cm^2, while August represented the greatest difference within a month, varying by 1.5 $\log_{10}$ CFU/cm^2 over the years sampled. Seasonal trends in microbial contamination have been reported (Rigobelo *et al.* 2006; Ruby *et al.* 2007; Rhoades *et al.* 2009). In general, across years in this study there was an increase in SPC from July to August, followed by a decrease through to October, before increasing over November and December, which was higher than the rise in August. Although the exact mechanism influencing these seasonal trends is poorly understood, temperature and rainfall have been implicated in other studies.

Whilst some studies reported higher shedding and prevalence of pathogens during warmer months (Rhoades *et al.* 2009; Williams *et al.* 2015; Cernicchiaro *et al.* 2020), seasonal trends have been less defined (Chapman *et al.* 1997) or not significant (Paiba *et al.* 2002) in areas where the mean daily maximum temperature difference between January and July is less variable, around 15°C compared to 30°C (Rhoades *et al.* 2009). In this study, contamination was high during August, September, and November before reaching a significant peak in December, with the lowest level occurring in March. January to July were not significantly different from each other with no clear low during winter as observed in other studies. Historical weather data reports a 13.9°C difference in mean daily maximum temperature between January and July at the location of this study (source: Bureau of Meteorology), indicating climatic similarity to other studies where seasonal effects are less pronounced and likely contributing to the absence of a comparably low contamination period over the winter months. Additionally, this study collected samples at a higher frequency over a longer period compared to other work, and may be more indicative of actual seasonal patterns.

Rainfall has been linked to pathogen shedding and carcase hygiene. Periods of high rainfall are related to both an increase in shedding (Lammers *et al.* 2015; Williams *et al.* 2015) and carcase detection (Rigobelo *et al.* 2006) of *E. coli*. The underlying mechanism for high rainfall increasing *E. coli* contamination is likely the resulting high environmental moisture content, which provides favourable conditions for bacterial growth and survival (Williams *et al.* 2005). Indeed, historical records from Australian processing reported a noticeable spike in bacterial loading on beef and sheep carcases from 2010 to 2013, following the break of the Millennial Drought which included a number of extreme rainfall events (Sumner *et al.* 2018). Climate data at the site of this study indicates July and August are the months of highest rainfall (60-70mm, source: Bureau of Meteorology), and predicted SPC for the following months, August and September, were higher compared to other months. The years 2016 and 2017 had an annual rainfall 177.2mm and 124.0mm, respectively, higher than the annual mean of 483.4mm, while 2019 was 125.4mm lower than average (source: Bureau of Meteorology). There was a large standard error in SPC during 2016 and 2017 which may be related to the high rainfall during these years. However, there was a clear trend for SPC to increase year-on-year during the period sampled, with a 1.5 $\log_{10}$ CFU/cm^2 increase between 2016 and 2020, thus factors other than rainfall are contributing to SPC counts at the year level.

Seasonal patterns of rainfall, also linked to pasture growth, have notably more effect on carcase microbiology in sheep than cattle (Sumner *et al.* 2018). This is likely due to the tendency for more small stock to be grazed exclusively on pasture compared to cattle, where up to 45% of slaughter stock are confinement fed on a grain ration (Meat and Livestock Australia 2020). While feed type alone did not affect carcase microbiology, the interaction between feed type and month was significant. For both grain and grass finished cattle, the highest SPC occurred during December, and the lowest in March. Grain finished cattle had a higher SPC during December and January, but lower values in February, March, April, July, and October. Overall, the SPC values for grain finished cattle were more variable than those for grass finished cattle, and variation in grain finished cattle SPC values may be contributing to the monthly effect recorded. Pre-slaughter, grain and grass finished cattle are managed similarly with up to two operators using hand-held high-pressure hoses to clean cattle during winter months, and sometimes no operators for high-pressure hoses during summer months such that they are not used, aligning with the hide cleanliness of arriving stock reported as being dirtier and cleaner at these times, respectively (J Phillips, personal communication). Grain finished cattle have notably dirtier hides during winter months following rainfall, and more staff are assigned to high-pressure hosing to improve visual cleanliness. Additionally, chain speed is slowed, and greater care taken during incisions, as extra washing often softens but does not completely remove hide contamination from grain finished cattle that are heavily dagged (Cernicchiaro *et al.* 2020). Winter months where these practices may occur include those where grain finished cattle had a lower SPC than grass finished cattle (June - July). Given the same operators and washing practices are applied to both feed types pre-slaughter, it is likely the extra measures applied to grain finished cattle during post-slaughter processing are contributing to the difference in SPC values between feed types. Furthermore, during summer months when less staff are assigned to washing, grain finished cattle have higher SPC values (December – January), which may result from less caution in post-slaughter processing compared to winter due to the visual improvement in hide cleanliness. While feed type is indicated as a key factor influencing visual hide cleanliness (Kiermeier *et al.* 2006), with higher contamination rates expected for grain finished cattle (Hughes 2001), animal cleanliness was not related to wash number or duration at this site during the months sampled (Chapter 4).

Feed type and animal husbandry influence further processing management decisions, including chiller loading. Carcases destined for specific markets related to feed type specifications and husbandry practices, such as Grain Fed Finished, Pasturefed Cattle Assurance System, European Union Cattle Accreditation Scheme, or processed as a service kill for a client, are loaded into chillers in contemporary groups. Chiller had a highly significant effect on carcase contamination, which has likely resulted from chiller loading patterns related to carcase specifications, rather than mechanical issues or faults of the infrastructure although these should not be disregarded. By loading carcases from animals of similar background together, it is logical that expected carcase contamination levels would be similar. Additionally, if cross-contamination of carcases occurs, it will likely be with the same bacteria at similar levels from the carcases in close proximity. Whilst mechanical issues resulting in an increase in chiller temperature could influence microbial counts, this is closely monitored and would have likely resulted in some significant interaction for chiller with day, month, or year, which was not the case. A time series analysis indicated no relationship in microbial contamination between days and weeks, suggesting that if a problem such a mechanical fault which changed chiller temperature occurred, it was rectified quickly and did not persist. Should a carcase return a result that was marginal or unacceptable, staff would have been more vigilantly monitoring hygiene practices in the days and weeks to follow to ensure results returned to the acceptable range and thus were not similar. A relationship in contamination levels between months and years was evident, supporting an effect of broad seasonal trends. Furthermore, synchronous patterns of *E. coli* O157 shedding in cattle from the same management groups have been reported (Lammers *et al.* 2015) which show similar contamination levels over time, but not necessarily day to day.

6.5 Conclusion

This study identified several factors associated with increased SPC at this plant, thus meeting the aims and providing useful information for planning future work which may impact carcase hygiene. Of the factors and their interactions tested, year, month, chiller, month by year, and month by feed type remained significant in the final multivariate analysis. Standard Plate Count levels increased year-on-year during the period of this study, though the reason for this was not clear. December had significantly higher contamination levels than all other months, and grain finished cattle were more contaminated than grass finished cattle at this time. The SPC of grain finished cattle had greater variability than pasture finished cattle throughout the year. Additionally, chillers four, and six to nine had carcases with less contamination, and this was likely related to chiller loading patterns. Future work investigating the effect of treatments on carcase hygiene should be planned to avoid December sampling to mitigate risk from existing higher contamination levels and commence initially with grass finished cattle, given their carcase hygiene is less susceptible to seasonal influences. Whilst factors increasing SPC have been identified, carcase contamination remained within acceptable limits, highlighting the excellent hygiene maintained at this plant.

Chapter 7 | General Discussion

7.1 Introduction

Australian beef commands a strong reputation for its high eating quality and hygienic standards. Factors that limit or result in unacceptable quality, or which impair hygiene, thus present a risk to trade and market access by decreasing supply. This thesis set out to understand the role of pre-slaughter factors in the production of high-quality Australian beef, specifically pre-slaughter hide washing practices and dark cutting. Pre-slaughter hide washing is a standard component of beef processing in Australia. However, as ubiquitous as this practice is, comparatively little research has been reported investigating its suitability and effectiveness, and even less so for any broader implications of this process. In this thesis, the impact of animal behaviours and environmental factors during the lairage period and their relationship to dark cutting incidence were examined, along with environmental factors in lairage and their effect on animal behaviour. The effect of pre-slaughter hide washing on animal behaviour was also reported in detail. In addition, this thesis afforded a greater understanding of factors related to the microbiological hygiene of beef carcases specific to the plant where this study was conducted.

7.2 Key findings

7.2.1 Meat quality

The initial motivation for this work arose from the problem of dark cutting beef and the comparatively little knowledge of abattoir based factors related to this condition. One of the main aims of this thesis was thus to identify abattoir based factors related to dark cutting beef, including environmental factors and animal behaviours. During lairage, data was recorded while cattle were unloading, in lairage pens, and whilst undergoing washing treatments. Environmental factors and animal behaviours were identified at each of these points in lairage which contributed to an increased dark cutting incidence, highlighting the importance of the pre-slaughter period to meat quality.

During unloading, the ramp used (old, new) did not affect dark cutting incidence ($P=0.968$), although animal behaviours and environmental factors during this process were significant ($P<0.05$, Table 3.8). The behaviours mounting, jumping, and exit score were associated with increased dark cutting incidence ($P<0.05$, Table 3.8). However, mounting was only observed in one group and caution should be used when considering this, to not over interpret the result without further observations of this behaviour during unloading. The association between exit score and animal temperament is reported (Vetters *et al.* 2013) and it was not surprising that animals with a more excitable temperament (higher exit score) were related to a higher dark cutting incidence (Table 3.8). The number of falls and slips during unloading were associated with a lower dark cutting incidence (Table 3.8), and this was a surprising result. Dark cutting incidence was found to increase as the number of stock on a truck and total time taken to unload increased (Table 3.8). The positive association between dark cutting incidence, and both unloading time and exit score, suggests that the longer unloading times were likely a result of excitable cattle also baulking and being difficult to move at unloading, rather than quieter animals (lower exit score) exiting slowly.

Cattle were observed during lairage with the most common behaviours recorded being vocalising and head down, which were recorded among 15-30% of a group (Figure 4.1). The only behaviour to significantly affect dark cutting incidence was head down behaviour on the morning of slaughter (P=0.038, Table 3.9). Each additional 1% of a group recorded with their head down was associated with a 0.33±0.16% increase in dark cutting incidence. Whilst only a small increase in percent of the group, this behaviour was observed on average among 14.8±1.2% of a group in the morning of slaughter (Figure 4.1), indicating potential for a larger increase in dark cutting incidence. Additionally, if head down recorded during washing results in a similar increase, there is potential for even higher levels of dark cutting with an average 29.8% of cattle in a group scored head down while being washed (Figure 5.3). Head down behaviour has been associated with increased dark cutting (Table 3.9), and this behaviour also increases during pre-slaughter washing treatments (Table 5.3, Figure 5.3), which may indicate the importance of the effects of washing on dark cutting (Table 3.10).

The number and duration of both lairage and high-pressure hose washes were recorded (Table 3.6). To our knowledge, this is the first time the extent of washing applied to cattle in a commercial processor has been reported, and this itself is useful information. Cattle received most washes in lairage, with an average of four washes lasting a total 64 minutes. An average of two high-pressure hose washes lasting a total 8 minutes were then applied. In lairage, more washes were associated with more dark cutting, but a longer duration was associated with a lower incidence of this condition (Table 3.10). This suggests the water being turned on initially was the source of stress and that cattle may begin to acclimatise over a longer duration as their environment becomes more predictable with continuous exposure to water. Initial analysis showed a clear relationship between lairage wash number and dark cutting incidence, however this effect was not as strong as more data were added. Although the observed effects of washing on dark cutting incidence were not consistent and changed during subsequent analyses, when considered together with the effects of washing on behaviour (Table 4.3, Table 5.3), pre-slaughter hide washing is without question a stressful procedure for cattle.

The number of high-pressure hose washes applied also had a significant effect on dark cutting incidence (P=0.03, Table 3.10), with additional washes associated with less dark cutting. The difference in the effect of high-pressure hosing on dark cutting may be occurring due to the difference in the time at which this practice occurs compared to lairage washing. High-pressure hosing occurs much closer to slaughter (within 20 minutes), compared to lairage washing which can begin hours before. Thus, high-pressure hosing may still be a stressor, but due to the point at which it occurs, lactic acid has not had time to clear from the muscle and will contribute to pH decline post-mortem. It is unlikely this form of washing is not a stressor, particularly when it's effect on behaviour is considered (Table 4.3), and more likely that muscle metabolism is masking the effect of stress.

7.2.2 Animal behaviour

Investigation of animal behaviour in lairage resulted in fruitful data, addressing our aim to understand factors impacting behaviour which could then be linked to animal welfare, with implications for meat quality as described above. Animal behaviour was observed during unloading, whilst resting in lairage pens, and during pre-slaughter washing procedures. This work is also the first study to describe behaviour of cattle in response to pre-slaughter hide washing in lairage (Chapter 5).

At unloading, slipping was observed among a higher portion of the group (7-15%) than any other behaviour (Figure 4.1). During this time behaviours exhibited were likely more reflective of animal temperaments, rather than resulting from facilities, as the unloading ramp used (new or old) did not have a significant effect on any behaviours (P>0.05, Table 4.1). In comparison, the time of day (afternoon or morning) did affect certain behaviours in lairage (Table 4.2). Vocalising, shaking, and laying were observed in a significantly higher (P<0.05) portion of animals during the morning, while agonistic interactions and head down were higher in the afternoon. The behaviours drinking and mounting, along with the group movement, score did not significantly differ between afternoon and morning (Table 4.2). Vocalisations also increased significantly (P<0.001) as high-pressure hose duration increased, and mounting increased with both the duration and number of times this treatment was applied (Table 4.3).

Behaviours across the areas of lairage recorded (unloading, lairage, washing) were not correlated (Figure 4.3), suggesting they could have been occurring in response to the environment. If behaviours were occurring because of group characteristics, such as a mob of heifers which might mount each other frequently, it is expected this behaviour would be observed at similar rates across all points of observation and thus would be correlated. However, the difference between periods supports behaviours occurring in response to environmental stimuli, which was highlighted during closer observation of behaviour during washing practices.

Animal behaviour changed in response to washing and did not return to pre-washing levels in the ten minute period immediately post-washing (Figure 5.2, Figure 5.3, Figure 5.4, Figure 5.5). Behaviours indicative of stress increased when washing in lairage pens (Figure 5.2, Figure 5.3) and with a high pressure hose (Table 4.3). In lairage, a greater portion of animals were observed with their head down or shaking once washing commenced (Table 5.2, Table 5.3), and an increase in the duration of high-pressure hose washes was associated with an increase in vocalising and mounting, the later which also increased with wash number

(Table 4.3). Once washing in lairage commenced, laying down behaviour almost completely ceased and did not return to the level observed prior to washing (Figure 5.4).

The behaviours recorded in this work are limited and do not represent every behaviour cattle exhibit during lairage. Rather, they were chosen either as indicators of stress, or as behaviours observed in healthy, unstressed animals. The behaviours indicative of stress have been linked to hormonal responses by other literature (Hemsworth *et al.* 2011; Park *et al.* 2017), providing an indication of physiological changes likely occurring at the time these behaviours were observed. Such changes include activation of the hypothalamic-pituitary-adrenal axis, resulting in an increase in secretion of stress hormones such as cortisol and subsequently glycogenolysis, which has the potential to affect meat quality (Tarrant 1989). The results of this work show time of day in lairage and pre-slaughter washing practices have the greatest effect on animal behaviour from the periods observed.

7.2.3 Carcase hygiene

The importance of pre-slaughter hide washing to the lairage period emerged as this work progressed, with its notable impacts on meat quality (Table 3.10) and animal behaviour (Table 4.3, Table 5.3). Subsequently, a controlled trial was planned (Appendix 1) which would test the effect of specified washing treatments on meat quality, animal behaviour, and meat hygiene, to further understand some of the results observed in this study. The proposed trial could not be completed within candidature due to complications from contracting for project funding and was further impeded by COVID-19, resulting in identification of an alternative piece of work. Historical data from the National Carcase Microbiology Monitoring program (NCMMP, previously the *E. coli* and *Salmonella* Monitoring Program) were collected and analysed with the aim to identify factors associated with increased carcase microbial contamination. Thus, the proposed trial could then be planned with consideration for this information, managing these factors accordingly and limiting any potential increase in carcase contamination.

Standard Plate Count (SPC), *Escherichia coli* and *Salmonella spp.* data were collected for this study, however only SPC was used for analysis due to the limited variation in *E. coli* and coliforms, and no positive *Salmonella* samples. Factors with a significant effect on carcase SPC were year, month, chiller, and the interactions between year and month, and month and feed type ($P<0.05$, Table 6.5). Contamination increased each year over the period sampled, and an autoregressive analysis identified a relationship between contiguous months and years sampled (Table 6.6), suggesting seasonal trends. Some patterns within months across years were also observed when examining the year and month interaction (Figure 6.4). Month and feed type formed another significant interaction, and the contamination for both grain and grass feed types was at its lowest in March and peaked in December (Figure 6.3). A larger range in SPC values across months were seen in grain finished cattle compared to grass finished cattle, which varied by 1.25 and 0.40 $\log_{10}$ CFU/cm^2, respectively. November through January, grain finished cattle had higher levels of contamination compared to other months, which may have resulted from less washing being applied when these animals arrive in a visually cleaner condition. Chiller also has a significant effect on contamination, with chillers 1, 2, 3, and 5 holding cattle with higher levels of contamination (Figure 6.2 D). This could be from mechanical or carcase loading issues in these chillers, or result from chiller loading patterns and carcase cross-contamination.

Grass finished cattle had more consistent levels of contamination throughout the year, suggesting they are less influenced by seasonal trends and making them a more suitable candidate initially for further studies than grain finished cattle from a risk mitigation perspective. Summer represented a period of lower contamination levels for grass finished cattle, indicative of a suitable time for sampling. However, this would need to be managed carefully with grass finished cattle supply, which tends to peak during spring following periods of high pasture growth. Chillers 6, 7, and 9 should be used with preference to avoid any confounding from chiller contamination issues which may be related to mechanical function, or limited space for appropriate carcase loading.

7.3 Industry implications

The results of this thesis, along with the scant previous literature have highlighted the need to evaluate current industry practices for pre-slaughter hide washing. The intricate relationship between washing, and meat quality and animal behaviour, demonstrates the necessity for a multifactorial approach to be taken when considering pre-slaughter hide washing given its broad reaching impacts. Australian Standards and International mandates require this practice to be continued for the foreseeable future, thus there will remain a need to wash Australian cattle prior to slaughter. With consideration for this and the known impacts of pre-slaughter hide washing, the acceptability and impact of continuing washing under current practices deserves review. Additionally, the implications of washing to processing with a broader perspective are considered, including indirect applications and wider impacts of these procedures, along with the need to prioritise an improvement in washing strategies for pasture finished cattle.

7.3.1 An ethical matrix approach

The ethical matrix is a tool designed to help guide decision making in relation to the acceptability and regulation of technologies (Mepham *et al.* 2006). This tool provides a structure to ethical deliberations using prima facie principles to consider respect for the wellbeing, autonomy, and fairness for all affected parties in relation to the issue in question. The interpretation of these principles used in the ethical matrix as they apply to each stakeholder group are outlined in Mepham *et al.* (2006). Briefly, wellbeing considers health and welfare, autonomy refers to freedom and choice, and fairness represents respect for justice. The ethical matrix structure has been adapted here to consider the continued use of current pre-slaughter hide washing practices using the results of this thesis in conjunction with published literature and personal experiences. A summary of this assessment is presented in Table 7.1 and the reasons guiding these outcomes are detailed below.

Table 7.1 An ethical matrix approach to considering the use of current pre-slaughter hide washing practices in Australia.

Respect for:	Wellbeing *Health, welfare*	Autonomy *Freedom, choice*	Fairness *Justice*
Producer	Negative	Negative	Negative
Processor	Negative	Positive	Negative
Wholesaler	Negative	Negative	Negative
Consumer	Inconclusive	Negative	Inconclusive
Animal	Negative	Negative	Negative
Environment	Negative	*	Negative

* Insufficient information available to consider this stakeholder and principle interaction

7.3.1.1 The Producer

Wellbeing: Producers care about the wellbeing of their livestock (Buddle *et al.* 2021) which may be impacted by washing (Chapter 5). While pre-slaughter washing practices do not occur on-farm, they may affect producer profits. Producers lose an estimated AUD$0.59 per kilogram of Hot Standard Carcase Weight (HSCW) on dark cutting carcases (Jose *et al.* 2015), which can be linked to increased washing (Chapter 3) (Petersen 1983).

Autonomy: Producers could implement management strategies on farm to improve the cleanliness of stock they consign and potentially minimise the amount of pre-slaughter washing applied to their cattle. However, they do not have the choice for their stock not to be subject to washing as this occurs at the discretion of processing staff. It is also the experience of the author, following presentations of the work from this thesis, that most producers are not aware their stock undergo washing whilst in lairage.

Fairness: Farmers do not have the option for their cattle not to be washed prior to slaughter if consigning product for domestic or international markets. Australian Standards and International trade mandates require stock to be presented for slaughter in a clean condition. Whilst these mandates only require cattle that are not acceptably clean to be washed, this practice is seemingly applied without exception.

7.3.1.2 The Processor

Wellbeing: Processors lose an estimated AUD$0.59 per kilogram of hot standard carcase weight on dark cutting carcases (Jose *et al.* 2015), which may be linked to increased washing (Chapter 3) (Petersen 1983). A significant amount of water is used during the washing process, along with electricity to run pumps providing water pressure and multiple labour units to apply washing treatments (Wockner and Jewell 2019).

Autonomy: Whilst cattle must be washed (Australia and New Zealand Food Regulation Ministerial Council 2007), the amount of washing to be applied and exactly what is considered clean is subjective, allowing processors control over the extent of the treatment. Additionally, recommendations around washing are minimal (Edge 2009; Animal Health Australia 2016), such that processors can select suitable methods tailored to their own facilities, and vary the application as required.

Fairness: Pre-slaughter washing is mandated by national and international standards and processors must ensure cattle are in a clean condition for processing (CODEX Alimentarius 2005; Australia and New Zealand Food Regulation Ministerial Council 2007). Post-slaughter methods to replace pre-slaughter washing may be investigated or in use by processors, however they must adhere to regulations for trade in both domestic and foreign markets and thus do not currently have the option not to apply washing treatments.

7.3.1.3 The Wholesaler

Wellbeing: If washing results in increased dark cutting, the amount of high-quality beef available is reduced. Product that is available will either increase in price, reducing wholesaler profit margins, or sales will be reduced due to price increases. Either outcome would thus impact business potential for the wholesaler.

Autonomy: Wholesalers and distributors do not have the option to purchase product from cattle that have not been subject to pre-slaughter hide washing treatments.

Fairness: Implications of pre-slaughter hide washing for meat quality are reported (2.5, 3.3.3). Additionally, the effectiveness of this practice at maintaining meat hygiene is questionable (2.4). Washing practices that result in meat quality defects or product not compliant with hygiene standards will reduce supply.

7.3.1.4 The Consumer

Wellbeing: The impact of pre-slaughter hide washing on meat hygiene is questionable, with some studies indicating washing increases microbial contamination (2.4). However, when used within a broader hygiene system, any negative effects may be countered or masked by the effectiveness of further strategies and interventions. Australia produces meat which is some 1-2 $\log_{10}$ lower in microbial counts compared to beef from other countries (Sumner *et al.* 2018), suggesting the system as a whole, including washing, does work in maintaining beef hygiene. The contribution of pre-slaughter washing practices to the overall hygiene is, however, questionable.

Autonomy: Consumers, who consider welfare when purchasing (Purslow 2017), do not have the choice to select meat from cattle that have not been subject to washing due to mandates on the practice. Furthermore, labelling does not include practices applied to cattle, including pre-slaughter washing, as such consumers are not able to make a fully informed choice when making a purchase.

Fairness: If washing results in increased dark cutting (Table 3.10), less high-quality product is available, increasing an already high price for Australian beef. Pasture finished cattle are particularly susceptible to dark cutting, and their beef attracts a premium price which could be pushed higher by limited supply.

7.3.1.5 The Animal

Wellbeing: Pre-slaughter hide washing results in behavioural changes indicative of stress (Table 4.3 Table 5.3), reduced muscle glucose and lactate concentration (Walker *et al.* 1999), along with an increased dark cutting incidence (Table 3.10) and high ultimate pH (Petersen 1983). These factors together suggest animals are experiencing washing as a stressor which is adversely affecting welfare, evidenced in these physiological and behavioural changes.

Autonomy: Cattle behaviour changes in response to washing and these changes can persist for at least ten minutes following the cessation of washing. Once washing commences, behaviours indicative of stress increase (Figure 5.2, Figure 5.3) and resting behaviours decrease (Figure 5.4) compared to pre-washing levels. The persistence of these changes during and following washing indicate the ability for cattle to express normal behaviour is restricted by the application of washing treatments.

Fairness: Pre-slaughter hide washing mandates value the needs of the consumer over the value of cattle as sentient beings. The priority of washing has been the improved cleanliness of slaughter stock to reduce human food-borne disease, without consideration for the experience of the animal undergoing the treatments.

7.3.1.6 The Environment

Wellbeing: A large volume of water is required for pre-slaughter washing (Wockner and Jewell 2019), which must subsequently be disposed of after use. There is thus potential to introduce the material being washed from the animal's hide, including contaminants like *E. coli* and *Salmonella*, into the environment during disposal.

Autonomy: There is insufficient information available to fairly consider any effect of washing on biodiversity. Understanding the impact of water used for washing entering the environment, and any subsequent effects on local flora and fauna, would aid consideration of this principle. For example, if *E. coli* or *Salmonella* washed from hides are redistributed to waterways, they may burden local biodiversity and result in population or species redistribution.

Fairness: The amount of water required for pre-slaughter washing has been estimated to be as high as 1ML per day in feed lot washing systems (Wockner and Jewell 2019). While there is opportunity for a large amount of this water to be from a recycled source, with treated water only required for a final spray, this water could otherwise be repurposed elsewhere. There are also additional energy requirements for maintaining and running water pumps and systems.

7.3.1.7 Summary

Following application of the principles of the ethical matrix to pre-slaughter hide washing with consideration for each stakeholder, the position of this author is that current practices for this procedure are not ethically acceptable. There is strong disregard for the animals enduring these procedures, which consequently affects the availability of quality product for consumers, along with the incomes of producers and processors. Additionally, there is a lack of freedom of choice around the application of washing, although processors can control the method and extent to which procedures are applied. Further research is required to completely appreciate the relationship between this practice and carcase hygiene, which impacts the availability of product for consumers, and the income for both producers and processors. From the information available, there are also negative impacts on the environment, particularly the amount of water used and its safe disposal.

7.3.2 Other effects of washing on processing

Whilst the anticipated purpose of pre-slaughter hide washing is to maintain carcase hygiene, its application in processing can also occur with additional outcomes intended. Presentation of cattle for slaughter in a damp or wet condition following washing is standard in Australia, and it is reported that this results in less dust or other airborne particles being released from the hide during processing. This effect may be greater depending on the type of cattle being processed, and the location from which they were consigned. For example, finer haired Bos indicus cattle tend to be less affected by dags than longer haired British or European types. Additionally, cattle from areas of lower rainfall may not carry dags to the extent of those from higher rainfall areas, but could have a greater degree of fine dust particles retained in the coat. Whilst not always completely removing contamination, washing can have a softening effect on large dags, and some slaughtermen believe a wet hide is easier to cut through and that knives do not blunt as quickly compared to when used on a dry hide.

7.3.3 The problem of pasture-finished cattle

Pasture finished cattle were the key focus of this work due to their high rates of dark cutting and the resulting impact on supply of premium beef through systems such as the Pasturefed Cattle Assurance System. Nationally, dark cutting incidence, defined by a pH greater than 5.71, peaked at 12.5% for pasture finished cattle and only 2.5% for grain finished cattle at the time of this work (Meat and Livestock Australia 2017). It should be noted that the true incidence of dark cutting for certified pasture finished cattle will be higher than this figure, given that any cattle which are not certified grain finished, are considered pasture finished in the aforementioned report.

Pasture finished cattle start from behind, the effects of which compound throughout production leaving them at a distinct disadvantage when it comes to meat quality potential. When compared to grain feeding, a pasture based diet has a lower metabolisable energy content, resulting in a less acidic rumen as there is no rapid fermentation like that which occurs in the digestion of grain. The production of propionate, the only precursor for gluconeogenesis, is favoured under the more acidic rumen conditions produced by a grain diet, where its concentration can be double that produced from a pasture based diet. Consequently, the acetate to propionate ratio is higher and not as favourable for energy retention as a lower ratio, which results from a more acidic rumen environment. Production of glucose through this pathway is pivotal, as it provides 90% of ruminant glucose requirements (Nafikov and Beitz 2007). Thus, not only is there less metabolisable energy initially available for conversion to glycogen from a pasture diet, but rumen conditions are also less favourable to convert what energy is available to glycogen precursors.

The amount of muscle glycogen available at slaughter and contributing to pH decline is a function of the level of glycogenesis on-farm, which is influenced by nutrition, and any glycogen losses resulting from exposure to stressors during the pre-slaughter period. Different management practices in pasture and grain feeding systems likely result in different levels of acclimation to these stressors, where grain finished cattle have received greater exposure to transport (from farm to feedlot), the use of machinery, and both more and more frequent human-animal interactions. Such exposure decreases the likelihood these factors will activate the stress response and glycogenolysis during lairage for grain fed cattle. However, pasture finished cattle are more likely to perceive these factors as stressors due to their limited previous exposure, increasing the potential for glycogen breakdown. The effects of any glycogen loss are also more readily observable in pasture finished cattle due to the lower levels of glycogen initially available.

Management differences between feed systems can also influence the condition in which cattle are consigned for slaughter. Pasture finished cattle are generally cleaner, with more visual contamination seen on the hides of lot fed animals. Consequently, pasture finished cattle should only require little, if any, washing in comparison to grain finished cattle. The effect of feed type on visual cleanliness has been related to the type of dung produced, with contamination more likely from animals with wet dung, typically resulting from a grain diet. Additional washing of pasture finished cattle to the extent applied to grain fed animals is excessive, and contributing to increased dark cutting incidence unnecessarily.

The higher incidence of dark cutting in pasture finished cattle, resulting from limited glycogen stores which are more readily impacted by stressors, can also contribute to further meat quality issues. Dark cutting beef is more prone to microbial spoilage as the higher pH (nearing pH 7.0) provides more favourable conditions for bacterial growth. The work herein reported higher-than-average SPC compared to those recorded nationally, which may be impacted by the high levels of dark cutting also observed.

Whilst a pasture finished diet presents many challenges for meat quality, strategies can be implemented to manage these and maintain supply of high quality pasture-finished beef. The outcomes of uninformed management based on a deficit in literature have been reported in the results of this thesis, and contemplation of the ethical acceptability of current washing practices has identified significant room for improvement. In looking to the future and further work in this area, it is evident that pre-slaughter management of pasture finished cattle must be addressed as a priority.

7.4 Recommendations

7.4.1 Future research

The literature surrounding pre-slaughter hide washing is scant, leaving significant opportunity for further research to broaden our knowledge. A research priority in this area is investigation of the effects of specified washing treatments on meat quality, meat hygiene, and animal welfare, coalescing to provide a complete picture of the extent of the impacts of this practice. No studies to date have been comprehensive across these areas and given there is much variation in the washing methodologies used between studies (2.2), a direct comparison or compilation of results is difficult.

A study considering the effects of pre-slaughter hide washing on meat quality, meat hygiene, and animal welfare has been proposed and accepted for funding (Appendix 1). Briefly, this trial will recruit 250 replicate groups of 20 cattle, each assigned one of fifteen washing treatments. Treatments will vary in the type and number of washes, along with the time prior to slaughter which they are applied. Mobs will be video recorded prior to, during, and following washing to allow video analysis of animal behaviour. Microbiological samples will be collected and analysed from five random carcases within each replicate group. All carcases will be Meat Standards Australia graded and a muscle sample will be collected to determine muscle glycogen concentration. This sampling was designed to test with a power of 99% at a 0.01 level of significance to detect a difference of one standard deviation (0.76 $\log_{10}$ CFU/cm^2) in microbial loading. On completion, this work will allow identification of a method for washing, from those combinations tested, that maintains or improves carcase hygiene, without having a negative effect on meat quality or animal behaviour.

Future work in which meat quality is a focus would benefit from use of a linear measure, such as glycogen concentration, to increase the sensitivity of analysis. Binomial data for meat quality as used in this thesis, with carcases defined as either a dark cutter or not, is limited in ability to identify trends and the extent to which glycogen depletion may be occurring. For instance, a treatment may increase glycogen depletion and limit pH decline, but not to the extent the carcase is graded a dark cutter. Identifying this type of trend would be valuable as the meat quality outcome could be different in animals with glycogen reserves that are lower initially, such as pasture finished cattle. Such measures would also provide a physiological indicator of the animal's welfare through its response to treatments, which may not be reflected in behaviours. Additional attributes of meat quality worth consideration in relation to washing are bruising and ecchymosis. Wet floors from washing have the potential to result in animals slipping which may cause bruising, as could mounting behaviour, which increases in response to washing (Table 4.3). While the exact mechanisms and causes of ecchymosis are not clear, it is reported in response to pre-slaughter stressors and thus washing may contribute to the presence of this condition in some animals.

Further examination of the effect of washing on hide and carcase microbial contamination, and the relationship between them, would also be beneficial. Whilst pre-slaughter hide washing aims to limit microbial contamination of the carcase and maintain food hygiene, there are many opportunities for contamination to be introduced, or for cross contamination to occur, along the production chain. Knives, processing equipment, operator hands, and adjacent carcases all present an opportunity for contamination after washing has occurred. Understanding the relationship between hide and carcase cleanliness in the system being investigated would thus be useful. The relationship between the hide and the carcase using artificially contaminated hides has been reported, but that from naturally occurring contaminants, and when using different washing methodologies, is unclear.

Animal behaviour data greatly complemented meat quality data in this study, providing useful insights which were less apparent when considering either set of data alone. Where possible, the continued exploration of animal behaviour data would thus be beneficial to future work in this area. This study explored animal behaviour in lairage, including while cattle were being washed, investigating differences in behaviour between groups and the treatments they received. A more informative behaviour measure might actually be the difference in behaviour of a particular group on farm and in lairage. Animal temperament, past experience and environment will result in behavioural differences between groups. Thus, a group recorded vocalising a large amount in lairage might just be a group that is usually very vocal, and so a high level of vocalisation would be expected. However, if a group does not normally use vocal signalling within their group, and vocalisations increase during lairage, then this might be more indicative of a stressor. While baseline resting behaviours in lairage were recorded in this study for comparison, these may still differ from typical group behaviour and not represent true resting behaviour. Whilst informative, behaviour work as described above would be very difficult to capture without a close relationship with producers, including knowledge of when and where cattle are to be processed, and such, ability to access processing facilities.

Additional work that would contribute to our understanding of cattle behaviour in lairage includes the use of behavioural ethograms. Our understanding of what constitutes normal cattle behaviour during lairage is lacking but would provide useful points of comparison for observed behaviours. Ethograms consider all behaviours an animal performs, allowing creation of a time budget detailing duration spent performing every behaviour, rather than scan or continuous sampling for specified behaviours such as the methodology used in this work. Use of an ethogram would require video recording to enable observations of an entire mob and subsequent video analysis which would be time consuming. However, the understanding of group behaviour and how cattle allocate their time in general, including when exposed to stressors, may afford better interpretation of behavioural data. The overall portion of an animal's time spent performing behaviours indicative of stress could provide more information than simply the portion of a group performing a specific behaviour linked to stress.

Understanding of normal group behaviours may not be particularly useful from a day-to-day management perspective in lairage, but being able to identify behaviours which are not normal and occurring in response to stress would aid in the identification of stressors in the lairage environment that could subsequently be changed or managed. Another consideration for behaviour work is the use of control animals. The work detailed herein used groups as their own control, however environmental changes additional to washing at the time of recording may have elicited behaviour changes. The use of a control group, recorded at the same time as other groups are being washed, would help account for the effect of any additional environmental changes, such as noisy machinery, staff walking past pens, or stock movement.

Other work which would add to our knowledge is an understanding of physiological parameters (such as blood cortisol, muscle glycogen and lactate concentration) and their response to washing. This would be particularly useful for understanding the effect of prolonged exposure to washing practices, and help us understand if acclimatisation is occurring, where animals become less responsive to perceived stressors over time. This could suggest the exact number of washes applied is less important than the overall extent of washing. For example, if a small number of less than three washes is applied, compared to high number of more than five washes. The effect of washing on physiological parameters likely plateaus at a certain point where hormone secretion can no longer increase, although the exposure to and perception of stress continues. Glycogen breakdown may therefore be initiated and begin at a rapid rate before slowing after an extended period of exposure following other physiological changes. Such work would aid our interpretation of the different effects of time reported in this work, where a longer duration of lairage washing resulted in less dark cutting, where the average duration approached 2 hours (Table 3.6). However, we also observed that an increase in the duration of high-pressure hosing resulted in increased vocalisation (Table 4.3), indicating stress, but comparatively lower rates of dark cutting (Table 3.10). In comparison, this treatment was only applied for approximately 20 minutes and occurred about 10 minutes prior to slaughter, potentially allowing glycogen breakdown to be initiated as a stress response is mounted, but not providing enough time for glycogen to be cleared from the muscle, and thus contributing to lowering ultimate pH.

Investigation of the impact of washing on body temperature would also be of benefit when considering effects on animal welfare and meat quality. A thermal imaging camera would enable body surface temperature of animals within a group to be easily measured before, during, and after washing procedures. Other studies have reported the effective use of water sprays to lower body temperature of dairy cattle under hot climatic conditions. It follows that body temperature could also be lowered in cold conditions, such as winter months when cattle appear visually dirtier and are washed more, compared to summer months. Additionally, much washing occurs prior to slaughter commencing, from as early as 4am, when temperatures are typically lower than later in the day. If washing results in a lowering of body temperature in already cold climatic conditions, this will likely result in actions such as shivering, postural changes, and mobilisation of energy in order to maintain body temperature (5.4). Thus, washing may have further implications on animal welfare to those already described, and actions the animal may take to compensate for thermal discomfort could result in energy use, including the mobilisation of glycogen, with the potential to increase the likelihood of dark cutting.

Future work should also seek to investigate the impact of pre-slaughter washing on wider aspects of production, including trim loss. To maintain food hygiene, carcases are subject to numerous points of inspection and trimming throughout processing to remove visual contaminants. Literature has shown the effectiveness of pre-slaughter washing at improving visual cleanliness of both the hide and carcase, where carcases from unwashed animals have greater visual contamination than those from washed animals, thus more trimming is likely to occur. The extent of trimming and difference between washed and unwashed carcases is an important consideration for yield loss, and thus profit, when assessing the use of washing practices from a broad perspective.

7.4.2 Industry recommendations

Whilst there is much opportunity for further work in the area of pre-slaughter washing, the available literature and results of this thesis provide grounds for clear actions that should be implemented by industry to refine practices and ensure any detrimental effects of washing are minimised. An immediate action with several applications is to implement a cleanliness scoring system for cattle, similar to the UK Clean Cattle policy (Food Standards Agency 2002). Such a system using descriptive and photographic guides for cleanliness scoring would reduce the subjectivity of cleanliness assessment. An acceptable level of cleanliness can then be identified, below which treatments such as washing are not required. Results (4.4.4.2) suggest animal cleanliness changes minimally, if at all, once stock arrive at an abattoir, thus cattle could be assessed on arrival at unloading without concern that time spent in lairage will result in dirtier cattle. On arrival at processing, the cleanliness of stock should be inspected and washing treatments applied or limited accordingly, such that acceptably clean cattle do not receive excessive, unnecessary washing. For cattle not of an acceptable cleanliness, washing should only be applied for the maximum time necessary, and not a minute more, to improve cleanliness to an acceptable condition as defined by the scoring system. Cleanliness is subsequently improved to an acceptable standard for processing, meeting Australian and international mandates, but limiting prolonged exposure to treatments and any adverse effects on meat quality and welfare.

There is also a need to better communicate the importance of cleanliness to producers consigning stock for slaughter. Whilst transport can contribute to a decrease in cleanliness, the initial condition in which stock are loaded has a greater impact, thus there is an important role for producers to play. There is no clear method to maintain hide cleanliness of stock on farm, especially with challenges like the confinement of lot feeding, or wet winter pastures, although strategies such as the use of woodchip and sand pens for housing in the week prior to slaughter have been suggested. Producers appear to consider cleanliness of their stock as a problem for the processor. However, knowledge of the potential losses in profit from downgrading on dark cutters resulting from increased washing, and implications for animal welfare, would help producers value the importance of loading stock in a clean condition. Whilst producers receive a financial penalty in some European countries for consigning dirty stock, this is unlikely to be of benefit in Australian production systems which are larger in terms of stock numbers and more extensive in nature, and would likely only worsen tensions between processors and producers. Engaging producers will be key in working towards improving stock cleanliness and minimising the need for pre-slaughter washing.

7.5 Summary

Overall, the results of this work highlight the importance of the lairage period as a pivotal component of beef production, whilst contributing only a small portion of an animal's life, if not managed properly, the effects of this period can be irreversible and detrimental to the final meat product and negate the effort input to raising and managing animals on-farm. Meat quality and animal behaviour results indicated the importance of all aspects of lairage, from the time an animal is unloaded, during the resting period in lairage pens, and throughout pre-slaughter hide washing procedures. Whilst washing was originally introduced to improve carcase hygiene, it is clear its impacts are not limited to meat safety and that a broader approach considering its use is needed. Patterns and factors related to increased microbiological carcase contamination were identified, providing guidance for such future work to limit potential impacts on carcase hygiene when investigating best practice washing strategies.

Pre-slaughter hide washing is an undoubtedly complex issue with impacts far broader than its application intends. It is imperative that the impact of washing on carcase hygiene is understood, particularly given its requirements under national and international mandates. Literature does not clearly indicate washing is an effective strategy to maintain carcase hygiene, and there are negative effects of washing on meat quality and animal behaviour. Additionally, pre-slaughter hide washing is one more factor contributing to the problem of dark cutting beef. Improving washing practices by managing their impact on dark cutting will reduce or remove this additional source of glycogen loss, but it will not solve this highly multifactorial problem. However, if vulnerable cattle such as those from pasture finished systems with limited glycogen stores are on the verge of cutting dark, then improved washing practices have a role to play in ensuring they can be MSA graded.

Washing prior to slaughter has been an accepted practice to date and has formed a standard component of antemortem management. However, it cannot continue without refinement to ensure it is not occurring to the detriment of overall meat quality. This thesis has highlighted an important area for further work to understand the best practice for animal management during lairage, an unequivocally significant period in the production of high quality Australian beef.

| References

Agricultural Resource Management Council of Australia and New Zealand, A (1995) 'Australian Standard for construction of premises processing animals for human consumption.' (CSIRO Publishing: Collingwood, Victoria)

Aiyegoro, OA (2014) MICROBIAL CONTAMINATION | Microbial Contamination of Fresh Meat. In 'Encyclopedia of Meat Sciences (Second Edition).' (Eds M Dikeman, C Devine.) pp. 285-288. (Academic Press: Oxford)

Albright, JL, Arave, CW (1997) 'The behaviour of cattle.' (CAB International: Wallingford, Oxon, UK)

Animal Health Australia (2016) 'Australian Animal Welfare Standards and Guidelines for Cattle.' (Australian Government Department of Agriculture:

AUS-MEAT, 2018. Beef and veal language categories. https://www.ausmeat.com.au/WebDocuments/Beef_and_Veal_Language_Categories.pdf.

Australia and New Zealand Food Regulation Ministerial Council, 2007. Australian Standard for the hygienic production and transportation of meat and meat products for human consumption. CSIRO Publishing, Collingwood, Victoria. AS 4696:2007:

Australian Quarantine and Inspection Service (Ed. Technical Services Branch (1995) 'Operational guidelines for the welfare of animals at abattoirs and slaughterhouses.' (Australian Government Publishing Service: Canberra, Australia)

Ayres, J (1955) Microbiological Implications in the Handling, Slaughtering, and Dressing of Meat Animals. *Advances in food research* **6**, 109-161.

Bacon, RT (2005) 16 - Physical decontamination strategies for meat. In 'Improving the Safety of Fresh Meat.' (Ed. JN Sofos.) pp. 318-349. (Woodhead Publishing:

Bacon, RT, Belk, KE, Sofos, J, Clayton, RP, Reagan, JO, Smith, GC (2000) Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *Journal of Food Protection* **63**, 1080-1086.

Barco, L, Belluco, S, Roccato, A, Ricci, A (2015) A systematic review of studies on Escherichia coli and Enterobacteriaceae on beef carcasses at the slaughterhouse. *International Journal of Food Microbiology* **207**, 30-39.

Barnett, JL, Hemsworth, PH (1990) The validity of physiological and behavioural measures of animal welfare. *Applied Animal Behaviour Science* **25**, 177-187.

Bell, R (1997a) Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology* **82**, 292-300.

Bell, RG (1997b) Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology* **82**, 292-300.

Biss, ME, Hathaway, SC (1995) Microbiological and visible contamination of lamb carcasses according to preslaughter presentation status: implications for HACCP. *Journal of Food Protection* **58**, 776 - 783.

Biss, ME, Hathaway, SC (1996) Effect of pre-slaughter washing of lambs on the microbiological and visible contamination of the carcases. *Veterinary Record* **138**, 82-86.

Bonny, SPF, O'Reilly, RA, Pethick, DW, Gardner, GE, Hocquette, J-F, Pannier, L (2018) Update of Meat Standards Australia and the cuts based grading scheme for beef and sheepmeat. *Journal of Integrative Agriculture* **17**, 1641-1654.

Borch, E, Kant-Muermans, M-L, Blixt, Y (1996) Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology* **33**, 103-120.

Brakel, WJ, Leis, RA (1976) Impact of social disorganization on behavior, milk yield, and body weight of dairy cows. *J Dairy Sci* **59**, 716-21.

Buddle, EA, Bray, HJ, Ankeny, RA (2021) “Of course we care!“: A qualitative exploration of Australian livestock producers’ understandings of farm animal welfare issues. *Journal of Rural Studies* **83**, 50-59.

Burdick, NC, Randel, RD, Carroll, JA, Welsh, TH (2011) Interactions between Temperament, Stress, and Immune Function in Cattle. *International Journal of Zoology* **2011**, 373197.

Butler, D, Cullis, B, Thompson, R (2009) 'Mixed models for S language environments: ASReml-R Reference Manual.' (Department of Agriculture and Fisheries: Queensland)

Byrne, CM, Bolton, DJ, Sheridan, JJ, McDowell, DA, Blair, IS (2000) The effects of preslaughter washing on the reduction of *Escherichia coli* O157: H7 transfer from cattle hides to carcasses during slaughter. *Letters in Applied Microbiology* **30**, 142-145.

Cafe, LM, Robinson, DL, Ferguson, DM, McIntyre, BL, Geesink, GH, Greenwood, PL (2011) Cattle temperament: persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. *J Anim Sci* **89**, 1452-65.

Callaway, TR, Elder, RO, Keen, JE, Anderson, RC, Nisbet, DJ (2003) Forage feeding to reduce preharvest Escherichia coli populations in cattle, a review. *J Dairy Sci* **86**, 852-60.

Carpenter, CE, Cornforth, DP, Whittier, D (2001) Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Science* **57**, 359-363.

Cernicchiaro, N, Oliveira, AR, Hoehn, A, Noll, LW, Shridhar, PB, Nagaraja, TG, Ives, SE, Renter, DG, Sanderson, MW (2020) Associations Between Season, Processing Plant, and Hide Cleanliness Scores with Prevalence and Concentration of Major Shiga

Toxin–Producing Escherichia coli on Beef Cattle Hides. *Foodborne Pathogens and Disease*

Chapman, PA, Siddons, CA, Gerdan Malo, AT, Harkin, MA (1997) A 1-year study of Escherichia coli O157 in cattle, sheep, pigs and poultry. *Epidemiol Infect* **119**, 245-50.

Chen, JM, Schütz, KE, Tucker, CB (2015) Cooling cows efficiently with sprinklers: physiological responses to water spray. *Journal of Dairy Science* **98**, 6925 - 6938.

Chen, JM, Schütz, KE, Tucker, CB (2016a) Cooling cows efficiently with water spray: behavioural, physiological, and production responses to sprinklers at the feed bunk. *Journal of Dairy Science* **99**, 4607 - 4618.

Chen, JM, Schütz, KE, Tucker, CB (2016b) Sprinkler flow rate affects dairy cattle avoidance of spray to the head, but not overall, in an aversion race. *Applied Animal Behaviour Science* **179**, 23 - 31.

CODEX Alimentarius, 2005. Code of hygienic practice for meat. CAC/RCP 58-2005:

Coombes, S, Gardner, G, Pethick, D, McGilchrist, P (2014) The impact of beef cattle temperament assessed using flight speed on muscle glycogen, muscle lactate and plasma lactate concentrations at slaughter. *Meat science* **98**, 815-821.

Dantzer, R, Mormède, P (1983) Stress in Farm Animals: A Need for Reevaluation. *Journal of Animal Science* **57**, 6-18.

Davies, MH, Webster, SD, Hadley, PJ, Stosic, PJ (2000) Production factors that influence the hygienic condition of finished beef cattle. *Veterinary Record* **146**, 179-183.

Department of Agriculture Water and the Environment, 2020a. Approved Methods for Microbiological Testing of Meat and Meat Products. https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/biosecurity/export/meat/elmer-3/approved-methods-microbiological-testing.pdf.

Department of Agriculture Water and the Environment (2020b) Microbiological manual for sampling and testing of export meat and meat products.

Dunn, CS (1990) Stress reactions of cattle undergoing ritual slaughter using two methods of restraint. *The Veterinary record* **126**, 522-525.

Edge, MK (2009) National animal welfarre standards for livestock processing establishments. (Meat and Livestock Australia [Accessed

Egan, AF, Ferguson, DM, Thompson, JM (2001) Consumer sensory requirements for beef and their implications for the Australian beef industry. *Australian Journal of Experimental Agriculture* **41**, 855-859.

Elder, RO, Keen, JE, Siragusa, GR, Barkocy-Gallagher, GA, Koohmaraie, M, Laegreid, WW (2000) Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Procedings of the National Academy of Sciences of the United States of America* **97**, 2999-3003.

Ferguson, DM, Gerrard, DE (2014) Regulation of post-mortem glycolysis in ruminant muscle. *Animal Production Science* **54**, 464-481.

Ferguson, DM, Warner, RD (2008) Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Science* **80**, 12-19.

Fisher, AD, Verkerk, GA, Morrow, CJ, Matthers, LR (2002) The effects of feed restriction and lying deprivation on pituitary-adrenal axis regulation in lactating cows. *Livestock Production Science* **73**, 255-263.

Food Standards Agency (2002) Red Meat Safety and Clean Livestock. (Food Standards Agency: London, England). [Accessed

Gardner, GE, McGilchrist, P, Pethick, DW (2014) Ruminant glycogen metabolism. *Animal Production Science* **54**, 1575-1583.

Ghafir, Y, China, B, Dierick, K, De Zutter, L, Daube, G (2008) Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. *J Food Prot* **71**, 35-45.

Gill, CO (2004) Visible contamination on animals and carcasses and the microbiological condition of meat. *Journal of Food Protection* **67**, 413-419.

Gonyou, HW, Christopherson, RJ, Young, BA (1979) Effects of cold temperature and winter conditions on some aspects of behaviour of feedlot cattle. *Applied Animal Ethology* **5**, 113-124.

Grandin, T (1997) Assessment of stress during handling and transport2. *Journal of Animal Science* **75**, 249-257.

Grandin, T (1998) The feasibility of using vocalization scoring as an indicator of poor welfare during cattle slaughter. *Applied Animal Behaviour Science* **56**, 121-128.

Grandin, T (2000) Effect of animal welfare audits of slaughter plants by a major fast food company on cattle handling and stunning practices. *J Am Vet Med Assoc* **216**, 848-51.

Grandin, T (2007) 'Livestock handling and transport.' (CABI: Cambridge, MA)

Grandin, T (2010) Auditing animal welfare at slaughter plants. *Meat Science* **86**, 56-65.

Grau, FH (1979) Fresh meats - bacterial association. *Archiv fur Lebensmittelhygiene* **30**, 87-92.

Grau, FH, Smith, MG (1974) Salmonella Contamination of Sheep and Mutton Carcasses Related to Pre-slaughter Holding Conditions. *Journal of Applied Bacteriology* **37**, 111-116.

Greenwood, P, House, J, Brunner, J, Rowland, D, Whitehouse, J, Ferrier, G (2000) Effects of pre-slaughter cleaning techniques and handling on indices of stress in cattle, and associations with some meat characteristics. *Asian-Australian Journal of Animal Science* **13**, 210.

Greiveldinger, L, Veissier, I, Boissy, A (2009) Behavioural and physiological responses of lambs to controllable vs. uncontrollable aversive events. *Psychoneuroendocrinology* **34**, 805-814.

Gygax, L, Siegwart, R, Wechsler, B (2007) Effects of space allowance on the behaviour and cleanliness of finishing bulls kept in pens with fully slatted rubber coated flooring. *Applied Animal Behaviour Science* **107**, 1-12.

Hadley, PJ, Holder, JS, Hinton, MH (1997) Effects of fleece soiling and skinning method on the microbiology of sheep carcases. *Veterinary Record* **140**, 570 - 574.

Hasegawa, N, Nishiwaki, A, Sugawara, K, Ito, I (1997) The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behavior and adrenocortical response. *Applied Animal Behaviour Science* **51**, 15-27.

Hauge, SJ, Nafstad, O, Røtterud, O-J, Nesbakken, T (2012) The hygienic impact of categorisation of cattle by hide cleanliness in the abattoir. *Journal of Food Control* **27**, 100-107.

Hemsworth, PH, Rice, M, Karlen, MG, Calleja, L, Barnett, JL, Nash, J, Coleman, GJ (2011) Human-animal interactions at abattoirs: relationships between handling and animal stress in sheep and cattle. *Applied Animal Behaviour Science* **135**, 24-33.

Hocquette, JF, Botreau, R, Legrand, I, Polkinghorne, R, Pethick, DW, Lherm, M, Picard, B, Doreau, M, Terlouw, EMC (2014) Win–win strategies for high beef quality, consumer satisfaction, and farm efficiency, low environmental impacts and improved animal welfare. *Animal Production Science* **54**, 1537-1548.

Honkavaara, M, Rintasalo, E, Ylönen, J, Pudas, T (2003) Meat quality and transport stress of cattle. *Deutsche Tierarztliche Wochenschrift* **110**, 125-128.

Hughes, J (2001) A system for assessing cow cleanliness. *In Practice* **23**, 517-524.

Hughes, JM, Clarke, FM, Purslow, PP, Warner, RD (2020) Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. *Comprehensive Reviews in Food Science and Food Safety* **19**, 44-63.

Hutchison, ML, Walters, LD, Avery, SM, Munro, F, Moore, A (2005) Analyses of Livestock Production, Waste Storage, and Pathogen Levels and Prevalences in Farm Manures. *Applied and Environmental Microbiology* **71**, 1231-1236.

Immonen, K, Ruusunen, M, Hissa, K, Puolanne, E (2000) Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. *Meat Sci* **55**, 25-31.

Jacob, RH, Gardner, GE, Pethick, DW (2009) Repletion of glycogen in muscle is preceded by repletion of glycogen in the liver of Merino hoggets. *Animal Production Science* **49**, 131-138.

Jose, CG, McGilchrist, P, Perovic, JL, Gardner, GE, Pethick, DW (2015) The economic impact of dark cutting beef in Australia. In 'International Congress of Meat Science and Technology. Clermont-Ferrand, France'.

Kannan, G, Jenkins, A, Eega, K, Kouakou, B, McCommon, G (2007) Preslaughter spray-washing effects on physiological stress responses and skin and carcass microbial counts in goats. *Journal of Small Ruminant Research* **67**, 14-19.

Kendall, PE, Verkerk, GA, Webster, JR, Tucker, CB (2007) Sprinklers and shade cool cows and reduce insect-avoidance behaviour in pasture-based dairy systems. *Journal of Dairy Science* **90**, 3671 - 3680.

Kenny, FJ, Tarrant, PV (1987) The behaviour of young Friesian bulls during social re-grouping at an abattoir. Influence of an overhead electrified wire grid. *Applied Animal Behaviour Science* **18**, 233-246.

Kenny, FJ, Tarrant, PV (1988) The effect of oestrus behaviour on muscle glycogen concentration and dark-cutting in beef heifers. *Meat Sci* **22**, 21-31.

Keys, JE, Smith, LW, Weinland, BT (1976) Response of dairy cattle given a free choice stall location and three bedding materials. *Journal of Dairy Science* **59**, 1157-1162.

Kiermeier, A, Bobbitt, J, Vanderlinde, P, Higgs, G, Pointon, A, Sumner, J (2006) Use of routine beef carcase Escherichia coli monitoring data to investigate the relationship between hygiene status of incoming stock and processing efficacy. *International journal of food microbiology* **111**, 263-269.

Kilgour, RJ (2012) In pursuit of “normal”: A review of the behaviour of cattle at pasture. *Applied Animal Behaviour Science* **138**, 1-11.

Knee, B, Cummins, L, Walker, P, Warner, R (2004) Seasonal variation in muscle glycogen in beef steers. *Australian Journal of Experimental Agriculture* **44**, 729-734.

Knee, BW, Cummins, LJ, Walker, PJ, Kearney, GA, Warner, RD (2007) Reducing dark-cutting in pasture-fed beef steers by high-energy supplementation. *Australian Journal of Experimental Agriculture* **47**, 1277-1283.

Knights, M, Smith, GW (2007) Decreased ACTH secretion during prolonged transportation stress is associated with reduced pituitary responsiveness to tropic hormone stimulation in cattle. *Domest Anim Endocrinol* **33**, 442-50.

Kondo, S, Kawakami, N, Kohama, H, Nishino, S (1984) Changes in activity, spatial pattern and social behavior in calves after grouping. *Applied Animal Ethology* **11**, 217-228.

Kutzer, T, Steilen, M, Gygax, L, Wechsler, B (2015) Habituation of dairy heifers to milking routine—Effects on human avoidance distance, behavior, and cardiac activity during milking. *Journal of Dairy Science* **98**, 5241-5251.

Lammers, GAC, McConnel, CS, Jordan, D, Ayton, MS, Morris, S, Patterson, EI, Ward, MP, Heller, J (2015) Synchronization of E. coli O157 shedding in a grass-fed beef herd: a longitudinal study. *Epidemiology and Infection* **143**, 3244-3255.

Lauber, MCY, Hemsworth, PH, Barnett, JL (2006) The effects of age and experience on behavioural development in dairy calves. *Applied Animal Behaviour Science* **99**, 41-52.

Lawrie, RA, Ledward, D (2014) 'Lawrie's Meat Science.' (Elsevier Science: Burlington)

Legrand, A, Schütz, KE, Tucker, CB (2011) Using water to cool cattle: behavioural and physiological changes associated with voluntary use of cow showers. *Journal of Dairy Science* **94**, 3376 - 3386.

Loudon, KMW, Lean, IJ, Pethick, DW, Gardner, GE, Grubb, LJ, Evans, AC, McGilchrist, P (2018) On farm factors increasing dark cutting in pasture finished beef cattle. *Meat Sci* **144**, 110-117.

Lowman, B, Synge, B, Caldow, G (1997) Producing clean slaughter cattle. Edinburgh.

Malechek, JC, Smith, BM (1976) Behaviour of range cows in response to winter weather. *Journal of Range Management* **29**, 9-12.

María, GA, Escós, J, Alados, CL (2004) Complexity of behavioural sequences and their relation to stress conditions in chickens (Gallus gallus domesticus): a non-invasive technique to evaluate animal welfare. *Applied Animal Behaviour Science* **86**, 93-104.

María, GA, Villarroel, M, Chacón, G, Gebresenbet, G (2004) Scoring system for evaluating the stress to cattle of commercial loading and unloadind. *Veterinary Record* **154**, 818-821.

McEvoy, JM, Doherty, AM, Finnerty, M, Sheridan, JJ, McGuire, L, Blair, IS, McDowell, DA, Harrington, D (2000) The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology* **30**, 390-395.

McEvoy, JM, Doherty, AM, Sheridan, JJ, Thomson-Carter, FM, Garvey, P, McGuire, L, Blair, IS, McDowell, DA (2003) The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. *Journal of Applied Microbiology* **95**, 256-266.

McGilchrist, P, Perovic, J, Gardner, G, Pethick, D, Jose, C (2014) The incidence of dark cutting in southern Australian beef production systems fluctuates between months. *Animal Production Science* **54**, 1765-1769.

McGilchrist, P, Polkinghorne, RJ, Ball, AJ, Thompson, JM (2019) The Meat Standards Australia Index indicates beef carcass quality. *animal* **13**, 1750-1757.

Meat and Livestock Australia (2017) Australian beef eating quality insights 2017. North Sydney, NSW.

Meat and Livestock Australia (2019) 2019 Australian beef eating quality insights. Bowen Hills, Queensland.

Meat and Livestock Australia (2020) State of the industry report 2020: the Australian red meat and livestock industry. North Sydney, NSW.

Mepham, B, Kaiser, M, Thorstensen, E, Tomkins, S, Millar, K (2006) Ethical matrix manual. LEI, onderdeel van Wageningen UR.

Mies, PD, Covington, BR, Harris, KB, Lucia, LM, Acuff, GR, Savell, JW (2004) Decontamination of cattle hides prior to slaughter using washes with and without antimicrobial agents. *Journal of Food Protection* **67**, 579-582.

Miranda-de la Lama, GC, Leyva, IG, Barreras-Serrano, A, Pérez-Linares, C, Sánchez-López, E, María, GA, Figueroa-Saavedra, F (2012) Assessment of cattle welfare at a commercial slaughter plant in the northwest of Mexico. *Trop Anim Health Prod* **44**, 497-504.

Mølgaard, L, Damgaard, BM, Bjerre-Harpøth, V, Herskin, MS (2012) Effects of percutaneous needle liver biopsy on dairy cow behaviour. *Research in Veterinary Science* **93**, 1248-1254.

Nafikov, RA, Beitz, DC (2007) Carbohydrate and Lipid Metabolism in Farm Animals. *The Journal of Nutrition* **137**, 702-705.

Nottingham, PM, Penney, N, Harrison, JCL (1974) Microbiology of beef processing. *New Zealand Journal of Agricultural Research* **17**, 79-83.

O'Connell, J, Giller, PS, Meaney, W (1989) A Comparison of Dairy Cattle Behavioural Patterns at Pasture and during Confinement. *Irish Journal of Agricultural Research* **28**, 65-72.

Paiba, GA, Gibbens, JC, Pascoe, SJ, Wilesmith, JW, Kidd, SA, Byrne, C, Ryan, JB, Smith, RP, McLaren, M, Futter, RJ, Kay, AC, Jones, YE, Chappell, SA, Willshaw, GA, Cheasty, T (2002) Faecal carriage of verocytotoxin-producing Escherichia coli O157 in cattle and sheep at slaughter in Great Britain. *Vet Rec* **150**, 593-8.

Park, S, Kim, H, Piao, M, Jung, D, Kim, S, Na, S, Baik, M (2017) Effect of dehorning and multialleviation treatment on the growth, behavior, and blood parameters of Korean cattle bull calves. *Journal of Animal Science* 13-14.

Parola, F, Hillmann, E, Schütz, KE, Tucker, CB (2012) Preferences for overhead sprinklers by naïve beef steers: test of two nozzle types. *Applied Animal Behaviour Science* **137**, 13 - 22.

Petersen, GV (1983) The effect of swimming lambs and subsequent resting periods on the ultimate pH of meat. *Meat Science* **9**, 237-246.

Polkinghorne, R, Thompson, JM, Watson, R, Gee, A, Porter, M (2008) Evolution of the Meat Standards Australia (MSA) beef grading system. *Australian Journal of Experimental Agriculture* **48**, 1351-1359.

Polkinghorne, RJ, Thompson, JM (2010) Meat standards and grading: A world view. *Meat Science* **86**, 227-235.

Preston, FL (2015) Lairage factors affecting dark cutting beef. The University of Adelaide.

Preston, FL, Burnard, CL, Wilkes, MJ, Lee, SJ, Hazel, SJ, Pitchford, WS (2016) 'Pre-slaughter washing increases dark cutting incidence in beef, Australian Society of Animal Production.' Adelaide, Australia.

Preston, FL, Wilkes, MJ, McGilchrist, P, Pitchford, WS (2018) Pre-slaughter hide washing affects meat quality in pasture finished cattle. In '64th International Congress of Meat Science and Technology. Melbourne, Australia'.

Probst, JK, Neff, AS, Leiber, F, Kreuzer, M, Hilmann, E (2012) Gentle touching in early lide reduces avoidance distance and slaughter stress in beef cattle. *Applied Animal Behaviour Science* **139**, 42-49.

Purchas, RW, Aungsupakorn, R (1993) Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. *Meat Science* **34**, 163-178.

Purslow, PP (2017) Chapter 1 - Introduction. In 'New Aspects of Meat Quality.' (Ed. PP Purslow.) pp. 1-9. (Woodhead Publishing:

R Core Team (2020) 'R: A language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna, Austria)

Redbo, I, Ehrlemark, A, Redbo-Torstensson, P (2001) Behavioural responses to climatic demands of dairy heifers housed outdoors. *Canadian Journal of Animal Science* **81**, 9-15.

Rhoades, JR, Duffy, G, Koutsoumanis, K (2009) Prevalence and concentration of verocytotoxigenic Escherichia coli, Salmonella enterica and Listeria monocytogenes in the beef production chain: A review. *Food Microbiology* **26**, 357-376.

Rigobelo, EC, Stella, AE, Ávila, FA, Macedo, C, Marin, JM (2006) Characterization of Escherichia coli isolated from carcasses of beef cattle during their processing at an abattoir in Brazil. *International Journal of Food Microbiology* **110**, 194-198.

Romeyer, A, Bouissou, M-F (1992) Assessment of fear reactions in domestic sheep, and influence of breed and rearing conditions. *Applied Animal Behaviour Science* **34**, 93-119.

Ruby, JR, Zhu, J, Ingham, SC (2007) Using Indicator Bacteria and Salmonella Test Results from Three Large-Scale Beef Abattoirs over an 18-Month Period To Evaluate Intervention System Efficacy and Plan Carcass Testing for Salmonella. *Journal of Food Protection* **70**, 2732-2740.

Rushen, J (2008) 'The welfare of cattle.' (Springer: Dordrecht, Netherlands)

Sapolsky, RM, Romero, LM, Munck, AU (2000) How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions*. *Endocrine Reviews* **21**, 55-89.

Schirmann, K, Chapinal, N, Weary, D, Heuwieser, W, Von Keyserlingk, M (2011) Short-term effects of regrouping on behavior of prepartum dairy cows. *Journal of dairy science* **94**, 2312-2319.

Schrama, JW, Arieli, A, Brandsma, HA, Luiting, P, Verstegen, MW (1993) Thermal requirements of young calves during standing and lying. *Journal of Animal Science* **71**, 3285-3292.

Schütz, KE, Rogers, AR, Cox, NR, Webster, JR, Tucker, CB (2010) Dairy cattle prefer shade over sprinklers: effects on behaviour and physiology. *Journal of Dairy Science* **94**, 273 - 283.

Small, A, Buncic, S (2009) Potential for the cross-contamination of the hides of cattle while they are held in lairage. *Veterinary Record* **164**, 260-265.

Sonoda, Y, Oishi, K, Chomei, Y, Hirooka, H (2018) How do human values influence the beef preferences of consumer segments regarding animal welfare and environmentally friendly production? *Meat Science* **146**, 75-86.

Standards Australia, 2020. Livestock loading/unloading ramps and forcing pens. Standards Australia, AS 5340:2020:

Stephens, DB, Toner, JN (1975) Husbandry influences on some physiological parameters of emotional responses in calves. *Applied Animal Ethology* **1**, 233-243.

Stoye, S, Porter, MA, Stamp Dawkins, M (2012) Synchronized lying in cattle in relation to time of day. *Livestock Science* **149**, 70-73.

Sumner, J, Kiermeier, A, Jolley, J (2018) Microbiological food safety and storage life of Australian red meat. Australian Meat Processor Corporation.

Tao, S, Thompson, IM, Monteiro, APA, Hayen, MJ, Young, LJ, Dahl, GE (2012) Effect of cooling heat-stressed dairy cows during the dry period on insulin response. *Journal of Dairy Science* **95**, 5035 - 5046.

Tarrant, PV (1989) Animal Behaviour and Environment in the Dark-Cutting Condition in Beef - A Review. *Irish Journal of Food Science and Technology* **13**, 1-21.

Tennessen, T, Price, MA, Berg, RT (1985) The social interactions of young bulls and steers after re-grouping. *Applied Animal Behaviour Science* **14**, 37-47.

Terlouw, C (2005) Stress reactions at slaughter and meat quality in pigs: genetic background and prior experience: A brief review of recent findings. *Livestock Production Science* **94**, 125-135.

Thompson, J (2002) Managing meat tenderness. *Meat Science* **62**, 295-308.

Tresoldi, G, Schütz, KE, Tucker, CB (2018) Cooling cows with sprinklers: spray duration affects physiological responses to heat load. *Journal of Dairy Science* **101**, 4412 - 4423.

Troy, DJ, Kerry, JP (2010) Consumer perception and the role of science in the meat industry. *Meat Science* **86**, 214-226.

Tucker, CB, Rogers, AR, Verkerk, GA, Kendall, PE, Webster, JR, Matthers, LR (2007) Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter. *Applied Animal Behaviour Science* **105**, 1-13.

Tudor, G, Coupar, F, Pethick, DW (1996) Effect of silage diets on glycogen concentration in the muscle of yearling steers. *Proceedings of the Australian Society of Animal Production* **21**, 451.

Veissier, I, Boissy, A (2007) Stress and welfare: Two complementary concepts that are intrinsically related to the animal's point of view. *Physiology & Behavior* **92**, 429-433.

Vetters, MD, Engle, TE, Ahola, JK, Grandin, T (2013) Comparison of flight speed and exit score as measurements of temperament in beef cattle. *J Anim Sci* **91**, 374-81.

Vickers, KJ, Niel, L, Kiehlbauch, LM, Weary, DM (2005) Calf Response to Caustic Paste and Hot-Iron Dehorning Using Sedation With and Without Local Anesthetic. *Journal of Dairy Science* **88**, 1454-1459.

Von Keyserlingk, M, Olenick, D, Weary, D (2008) Acute behavioral effects of regrouping dairy cows. *Journal of Dairy Science* **91**, 1011-1016.

Walia, K, Lynch, H, Grant, J, Duffy, G, Leonard, FC, Lawlor, PG, Gardiner, GE (2017) The efficacy of disinfectant misting in the lairage of a pig abattoir to reduce *Salmonella* and *Enterobacteriaceae* on pigs prior to slaughter. *Journal of Food Control* **75**, 55-61.

Walker, P, Warner, R, Weston, P, Kerr, M (1999) 'Effect of washing cattle pre-slaughter on glycogen levels of M. smitendinosus and M. semimembranosus, International Congress of Meat Science and Technology.'

Ward, WR, Hughes, JW, Faull, WB, Cripps, PJ, Sutherland, JP, Sutherst, JE (2002) Observational study of temperature, moisture, pH and bacteria in straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds. *Veterinary Record* **151**, 199-206.

Warriss, PD, Brown, SN, Adams, SJ, Corlett, IK (1994) Relationships between subjective and objective assessments of stress at slaughter and meat quality in pigs. *Meat Sci* **38**, 329-40.

Watson, R, Polkinghorne, R, Thompson, JM (2008) Development of the Meat Standards Australia (MSA) prediction model for beef palatability. *Australian Journal of Experimental Agriculture* **48**, 1368-1379.

Watts, JM, Stookey, JM (2000) Vocal behaviour in cattle: the animal's commentary on its biological processes and welfare. *Applied Animal Behaviour Science* **67**, 15-33.

Wickham, H (2016) 'ggplot2: Elegant graphics for data analysis.' (Springer-Verlag New York:

Wigham, EE, Butterworth, A, Wotton, S (2018) Assessing cattle welfare at slaughter – Why is it important and what challenges are faced? *Meat Science* **145**, 171-177.

Williams, AP, Avery, LM, Killham, K, Jones, DL (2005) Persistence of Escherichia coli O157 on farm surfaces under different environmental conditions. *J Appl Microbiol* **98**, 1075-83.

Williams, KJ, Ward, MP, Dhungyel, OP, Hall, EJ (2015) Risk factors for Escherichia coli O157 shedding and super-shedding by dairy heifers at pasture. *Epidemiol Infect* **143**, 1004-15.

Wockner, K, Jewell, M (2019) Clean cattle manual. Meat and Livestock Australia, North Sydney, NSW.

Zavy, MT, Juniewicz, PE, Phillips, WA, VonTungeln, DL (1992) Effect of initial restraint, weaning, and transport stress on baseline and ACTH-stimulated cortisol responses in beef calves of different genotypes. *Am J Vet Res* **53**, 551-7.

Appendix 1 | PhD Research Proposal

The following research proposal was submitted as a Plant Initiated Project (PIP) jointly with Teys Australia to the Australian Meat Processor Corporation (AMPC). This PIP application was accepted and upgraded to a Core Program Project (Project No. 2019-1051). The contracting process for this project was lengthy, taking over 18 months from the time of initial submission for a contract to be finalised. Following this, complications from COVID-19 delayed this project further due to travel restrictions and site accessibility. Consequently, it was not feasible to complete this trial within the period of candidature.

The role of pre-slaughter hide washing in the production of high quality beef

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Overview

This project will determine the effect of pre-slaughter hide washing treatments on beef carcase contamination, meat quality, and animal behaviour and welfare. Cattle in Australia are required to be washed prior to slaughter under Australian Standards to minimise potential for carcase contamination. However, literature suggest that pre-slaughter hide washing is not consistently effective at controlling carcase contamination, and may in fact worsen it. The overall goal of this project is to determine an optimal method for pre-slaughter hide washing that controls or improves microbial carcase contamination, without having a negative effect on meat quality, or animal behaviour and welfare.

Project description

Currently, cattle in Australia are required to be washed prior to slaughter under Australian Standards, and those of importing countries, to minimise the potential for carcase contamination. However, literature suggest that at best, pre-slaughter hide washing is not consistently effective at controlling carcase contamination, and may in fact worsen it. Previous work has also shown pre-slaughter washing to have a negative effect on meat quality, increasing the incidence of dark cutting beef.

This project will follow pasture- and grain-finished cattle through lairage with a particular focus on pre-slaughter washing treatments. Mobs of cattle will be assigned a washing treatment, and the difference in microbial contamination, meat quality, and animal behaviour between treatments will be tested. Prior-to and during washing, animal behaviour will be observed as an indicator of discomfort and stress. Following washing and slaughter, a small muscle biopsy will be taken to determine muscle glycogen concentration and meat quality potential. Meat Standards Australia grading data will be collected on all carcases.

Additionally, carcases will be swabbed and tested for Standard Plate Counts, *E. coli*, and *Salmonella,* following the National Carcase Microbiological Monitoring Program procedures.

The information collected in this study will allow determination of an optimal pre-slaughter washing method which controls or improves carcase contamination, without negatively affecting meat quality or animal welfare. Refining washing methods may also decrease water consumption.

Project background and rationale

This project was initiated following an investigation into the high incidence of dark cutting beef observed in pasture finished cattle, and the lack of information regarding lairage factors related to this condition. A trial with the University of Adelaide was conducted at Teys Australia Naracoorte, finding an increase in the number of times cattle were washed prior to slaughter was associated with an increase in dark cutting incidence (Preston *et al*. 2018). The results of this study raised the question: why we are washing cattle?

Pre-slaughter washing is conducted under Commonwealth and international requirements. The 'Australian Standard for the hygienic production and transportation of meat and meat products for human consumption' (AS4696:2007) is the main regulation domestically, whilst the 'Code of hygienic practice for meat' (CAC/RCP58-2005) under the Codex Alimentarius sets the standard for meat hygiene internationally. These documents specify requirements for slaughter stock to be presented in a clean condition, and appropriate measures to be taken for animals presented in a compromised state of hygiene.

A literature review was conducted to assess the effectiveness of hide washing using water and identified a paucity of information around pre-slaughter washing practices. One study in cattle indicated an increase in carcase microbial contamination (SPC, coliforms, *E.coli, Salmonella*) following washing treatments (Mies *et al.* 2004), whilst another found washing had no effect on *E. coli* unless conducted for an extended period (Byrne *et al.* 2000). Similar results have been reported in sheep and goats, where, respectively, washing increased (Biss and Hathaway 1996) or had no effect (Kannan *et al.* 2007) on microbial contamination. The available literature do not provide support for pre-slaughter hide washing with water being an effective method for controlling carcase contamination.

Alternatives to pre-slaughter hide washing for improving carcase cleanliness could be worth consideration, particularly treatments conducted post-mortem. Improving hide cleanliness post-mortem would allow treatments to be applied which may be more effective, but unsuitable for use in the live animal. Such treatments might include the use of high-pressure washing, and the inclusion of antimicrobials at a level strong enough to improve cleanliness. This would also remove concern for meat quality issues arising due to animal stress, or the effect of treatments on animal behaviour. However, the implementation of such methods would require construction of new facilities along the kill floor chain and use extra space which is already limited. Our current proposal would use the existing infrastructure available widely in Australian processors to its maximum potential, thus not limiting implementation or uptake. By using existing facilities, once an effective method is determined, it could be taken up across industry almost immediately without any changes or additional infrastructure.

Biss, ME and Hathaway, SC (1996) 'Effect of pre-slaughter washing of lambs on the microbiological and visible contamination of the carcases.' *Veterinary Record,* 138:4, 82-86.

Kannan, G, Jenkins, AK, Eega, KR, Kouakou, B and McCommon GW (2007) 'Pre-slaughter spray washing effects on physiological stress responses and skin and carcase microbial counts in goats.' *Journal of Small Ruminant Research*, 67:1, 14-19.

Mies, PD, Covington, BR, Harris, KB, Lucia, LM, Acuff, GR and Savell, JW (2004) 'Decontamination of cattle hides prior to slaughter using washes with and without antimicrobial agents.' *Journal of Food Protection*, 67:3, 579-582

Preston, FL, Wilkes, MJ, McGilchrist, P and Pitchford, WS (2018) 'Pre-slaughter hide washing affects meat quality in pasture-finished cattle.' Paper presented at the *International Congress of Meat Science and Technology*, Melbourne, Australia, 12 - 17 August.

Project objectives

The objectives of this project are to:

1. Determine the effectiveness of pre-slaughter hide washing at controlling *microbiological contamination* on beef carcases
2. Determine the effect of pre-slaughter hide washing on *animal behaviour* and welfare
3. Determine the effect of pre-slaughter hide washing on beef *meat quality*
4. Develop an *optimal treatment* for pre-slaughter hide washing

Methodology

Animals required

- A minimum of 125 mobs of 40 head, comprising approximately 5000 total animals, will be recruited for this study, conducted over a minimum 24 days of sampling.
- Mobs from a vendor will be divided into lairage pens (wash groups) of up to 20 head (to not confound vendor with treatment) as per normal lairage practice. These wash groups will form the unit of replication for the trial. Different washing treatments will be applied to each group, and subsequent analysis will be based around these treatments. No mobs will be mixed; larger mobs (e.g. 40+ head) will be divided into smaller groups of ~20 head, as per normal practice.

Microbiological sampling

- Cattle will be assessed for cleanliness on a 3 point scale on arrival (mud score 0; mud score 1-2; mud score 3-5), prior to allocation to a washing treatment group, to ensure groups are balanced.
- Washing treatments will be allocated a set duration and consist of a given number of possible washes by type. The treatment groups and their assigned number of washes for each wash type are shown in Table 1.
- The only groups to not receive any washing treatment will be group one and nine, and only mud score 0 animals will be allocated to these groups. This will ensure that all animals receiving a wash with untreated water also receive a wash with treated water.
- Treatments 1, 2, 3, 6, 8, 9, 10, 11, 13, 14, and 15 will be applied on both the afternoon of arrival and morning of slaughter to test differences in time of treatment application.

- To test for a difference of one standard deviation (0.76 $\log_{10}$ CFU/cm^2) in SPC between washing treatments with 99% power, 50 replicates per treatment are required when sampling at the 0.01 level of significance.
- If sampling is conducted on 5 carcases from each wash group from a mob, a minimum of 10 mobs is required per treatment for a power of 99% and 0.01 level of significance to detect a difference of one standard deviation (0.76 $\log_{10}$ CFU/cm^2)
- Thus, following slaughter, five carcases from each mob group, in a washing treatment group (*n* mobs =10), will be swabbed and sampled following standard NCMMP protocol, including testing. A total of 50 replicates per treatment, for a total of 1,200 replicates (5 carcases per wash group x 10 wash groups per treatment x 24 treatments (selected replicates morning and afternoon) = 1,200 replicates).
- These 1,200 samples will be analysed for SPC, coliforms, and *Salmonella.*

Animal behaviour

- Prior to-and-during washing treatments, animal behaviour will be observed and recorded.
- Video recording will be used for later analysis to allow all groups to be recorded and scored. Behaviour will be recorded the afternoon animals arrive, the morning of slaughter prior to washing treatments commencing, and during washing treatments and immediately following their cessation.
- Behaviours of interest include: drinking, mounting, vocalising, antagonistic interactions, shaking, head down, laying down, ruminating and group movement
- Behaviours will be analysed as the percent of the mob exhibiting a behaviour.

Meat quality

- MSA grading data will be collected and analysed at the treatment group level
- Dark cutting incidence and the effect of washing treatment will be determined from carcase grading data
- A small muscle biopsy will be taken from between the 12^{th} and 13^{th} rib on entry to the chiller to determine glycogen and lactate concentration at the point of slaughter, as a physiological measure of stress and indicator of potential for a carcase reaching a high ultimate pH.

Table 1 Washing treatment allocation based on cattle feed type and cleanliness score. The number and duration of each wash type (lairage, high-pressure hose, belly wash) and time of treatment application is also provided.

Treatment group	Feed type	Cleanliness score	Lairage wash (20 mins)	High-pressure hose (8 mins)	Belly Wash (1 min)	Including only am treatments	Including all pm treatments	Including select pm treatments*
1 – pasture, no wash#	Pasture	0 only	0	0	0	1	2	1*
2 – pasture, to cleanliness#	Pasture	0-5	As required^	As required^	1	1	2	2*
3	Pasture	0 – 5	0	0	1	1	2	2*
4	Pasture	0 – 5	0	1	1	1	2	1
5	Pasture	0 – 5	1	0	1	1	2	1
6#	Pasture	0 – 5	1	1	1	1	2	2*
7#	Pasture	0 – 5	1	2	1	1	2	1
8#	Pasture	0 – 5	5	1	1	1	2	2*
9- grain, no wash#	Grain	0 only	0	0	0	1	2	1*
10 – grain, to cleanliness#	Grain	0 – 5	As required^	As required^	1	1	2	2*
11#	Grain	0 – 5	1	1	1	1	2	2*
12#	Grain	0 – 5	1	2	1	1	2	1
13	Grain	0 – 5	5	0	1	1	2	2*
14#	Grain	0 – 5	5	1	1	1	2	2*
15	Grain	0 – 5	5	2	1	1	2	2*
Total treatment number						15	30	24
Total animal number						3,000	6,000	4,800
Total micro sample number						750	1,500	1,200

^ cattle washed until visible cleanliness is score 0; * select pm treatments to be included with all am treatments; # denotes treatments applied to both feed types

Project outputs and deliverables

- Identification of an optimal method for pre-slaughter hide washing, an industry standard practice conducted under requirements of the Australian Standards and those of export markets
- Improve the safety and hygiene of beef product by identifying the most suitable washing method for controlling microbiological carcase contaminants
- Decrease meat quality issues such as dark cutting beef, increasing the overall value of beef product produced
- Improve animal welfare by identifying washing methods which are least aversive to cattle
- Reduce water usage through optimisation of washing methods

Value proposition

Industry value

The current Meat Industry Strategic Plan identifies increasing global demand for quality red meat as the biggest opportunity and continued investment in efficiency and integrity through the supply chain as crucial. The biggest downside risk is consumer and community support. This specific project is a modest investment but targets the crucial interaction in the value chain between animal welfare, food safety and meat quality. The project fits within the AMPC sub-program 3 “Processing Hygiene, Quality and Meat Science”.

Australia is highly regarded internationally as suppliers of clean, green beef that is produced safely. This sentiment, which aids market access, is underpinned by good practices throughout our supply chains. However, literature suggest that pre-slaughter washing practices may have no effect, or in fact worsen carcase microbial contamination. Conducting this study and determining an effective methodology for pre-slaughter washing would help ensure the hygiene of our beef product, with potential to further improve it.

Producer and processor value

The project budget is $130,270 but the potential implications are large given that the outcome can be applied across multiple abattoirs. For the purposes of modelling relatively conservatively, it has been done for Naracoorte only, assuming the reduction of just 1 wash on all animals processed would lead to a drop in dark cutting rates of 1.2% which is approximately $2.16 per animal processed (1.2% x $0.60/kg x 300kg carcase) for the 100,000 processed annually. It was then assumed the benefit would start in 2020 with a 7% discount rate. The benefit in 2020 would be $200,880 ($2.16 x 100,000 – 7%) declining each year. The discounted benefit was then calculated over 5 years for a total benefit of $873,287 for an investment of $130,270 so the ROI would be 6.7:1. Clearly if the findings were adopted at multiple plants then this would rise. Also, we have only costed the benefit to the plant in reduced ark cutting, not the producer also and benefit to the plant in reduced labour and water use. That said, we have also not costed the in-kind contributions of Teys and University staff.

Consumer value

Consumers expect to purchase a product that is safe to consume and free of microbiological contamination. Shelf life is a key performance indicator for Australia's export-oriented meat value chain. Consumers are also becoming more concerned with the origins of their food and animal welfare, so it is important that procedures are conducted in a manner of highest welfare, minimising distress to the animal.