Towards the improvement of sheep welfare: Exploring the use of Qualitative Behavioural Assessment (QBA) for the monitoring and assessment of sheep

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AUTHOR’S DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institute.

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SUMMARY

Challenges faced by sheep in Australia in terms of disease, injury and management may compromise not only health and productivity but also welfare. These challenges represent a growing concern for both producers and the public. Hence there is an obvious need for the development of measures to allow producers, who may have limited access to stock or are constrained by time and/or resource availability, to monitor their sheep. There is a clear benefit to producers being able to readily identify animals whose welfare might be compromised and thus are in need of further care. However, the assessment of animal welfare is challenging under commercial conditions and to date, few measures are available to help producers recognise animals in compromised welfare states. Qualitative behavioural assessment (QBA) is an approach that captures the expressive behaviour of an animal, through the integration and summary of details of behavioural events, posture, and movement. In this way, QBA represents a valuable tool that offers insight into the physical and physiological aspects of animal welfare, and when used in conjunction with other key measures helps to provide a more complete and comprehensive picture of an animal's welfare state. Furthermore, QBA should be used together with other welfare measures, where it has been proposed to guide the interpretation of welfare data. As a welfare tool, QBA has been applied to assess the behavioural expression in numerous livestock species including pigs and cattle, however, this methodology is less well studied in sheep and more work is needed to validate QBA for practical application.

The aim of the research described in this thesis was to investigate whether the QBA methodology could be applied to assess the welfare of sheep subject to various welfare issues relevant to the Australian sheep industry. To this end, over four experimental chapters, QBA was applied to video footage captured of sheep in various states of compromised welfare, including those suffering from common injury and diseases; lameness, inappetence, flystrike, and gastro-intestinal parasitism, and those experiencing pain caused by routine husbandry procedures (ear tagging, castration, mulesing, and tail docking). Moreover, in two experimental chapters (Chapters 4 & 6), video footage was captured of sheep in positive welfare states (reduced gastro-intestinal parasite burden, and habituation to human presence). This video footage was also analysed quantitatively and other welfare measures including those of health/disease status, physical condition and locomotive activity were collected for validation purposes in each study.

Over four experimental chapters, it was demonstrated that observers, blind to experimental procedures and treatments, can reach a significant consensus in their interpretation and assessment of the behavioural expression of sheep, and that these assessments can relate meaningfully to the welfare state of the animal. In Chapter 3, observers were able to distinguish between flystruck and non-flystruck sheep using the QBA methodology, and the behavioural expression scores given to each sheep corresponded to the severity of strike and the condition of the wool. In Chapter 4, observers identified differences in the behavioural expression of sheep that related to the severity of gastro-intestinal parasitism (subclinical v. clinical). Moreover, it was discovered that the treatment of sheep to lessen gastro-intestinal parasite burden altered the behavioural expression of parasitised sheep. A significant consensus was also reached amongst observers in the assessment of lambs subject to routine husbandry procedures (ear tagging, castration, mulesing, and tail docking) in Chapter 5. Observers were able to distinguish lambs that were subject to these painful husbandry procedures and were administered either a placebo or analgesics (Tri-Solfen® and meloxicam), from the control lambs which were only restrained. Hence suggesting that the pain caused by these husbandry procedures alters the behavioural patterns and demeanour of lambs in a way that is identifiable to observers using the QBA methodology. Lastly, when observers viewed video footage of sheep traversing a walk-over-weigh (WoW) apparatus in Chapter 6, they were able to distinguish sheep that were either lame or habituated to the test apparatus and human presence, from the control animals. However, in this Chapter, observers were not able to distinguish between all treatment groups evaluated based on their behavioural expression, specifically differences in the demeanour of inappetent and control sheep was not evident, nor were observers able to distinguish between lame and habituated sheep.

In summary, the research presented in this thesis indicates that assessments of behavioural expression can be used under most of those conditions investigated to distinguish sheep in poor welfare states due to injury or disease, from those that are healthy. Furthermore, it appears that observers can reliably identify differences in behavioural expression related to positive welfare states. This work has detailed the behavioural expression of sheep as perceived by observers and has led to a greater understanding of the behavioural expression of sheep in different welfare states. It appears that through the assessment of demeanour or body language, QBA offers both relevant and valid assessments which may help producers gain an insight into the welfare state of their sheep. It is suggested that when used in conjunction with other select behavioural measures, QBA may represent a valuable tool for producers to improve the welfare of sheep in their care.

STATEMENT OF CANDIDATE CONTRIBUTION

The research presented in this thesis was undertaken from 2015-2020 at Murdoch University. Funding for this research was provided by the Sheep CRC and Murdoch University. This work is original and was carried out by myself, with contributions by my supervisory team and other collaborators that have been acknowledged for their assistance in the production of papers. Here, I detail the contributions of all authors towards each of the experimental chapters bound in this thesis.

Chapter 3 is predominantly my own work. I collected video footage of sheep, organised and edited video footage, organised and ran the QBA sessions, performed the quantitative behavioural scoring, assisted in collection of breech soiling parameters, undertook the statistical analysis, and drafted the manuscript. DWM assisted in the collection and editing of video footage, helped coordinate fieldwork and the QBA sessions, aided in quantitative behavioural scoring, and assisted in both the statistical analysis and in the reviewing and editing of the manuscript. PAF assisted in statistical analysis and in the reviewing and editing of the manuscript. FA, ALB and SLW assisted in reviewing and editing the manuscript. All authors helped conceive the study, contributed to the experimental design, and approved manuscript for publication. This chapter has been published in Animals.

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Chapter 4 is predominantly my own work. I collected video footage of sheep, organised and edited video footage, organised and ran the QBA sessions, collected locomotive parameters, assisted in the collection of production parameters, faecal sampling and clinical evaluation of sheep, performed the faecal consistency scores, undertook the statistical analysis, and drafted the manuscript. DWM collected production parameters, assisted in the faecal sampling and clinical evaluation of sheep, helped coordinate fieldwork and the QBA sessions, helped collect locomotive parameters and video footage, and assisted with statistical analysis and in the reviewing and editing of the manuscript. ALB performed the clinical evaluation of the sheep, collected faecal samples, and assisted in the reviewing and editing of the manuscript. FA helped collect video footage and assisted in reviewing and editing the manuscript. PAF assisted in statistical analysis and in reviewing and editing the manuscript. SLW assisted in reviewing and editing the manuscript. All authors helped conceive the study, contributed to the experimental design, and approved manuscript for publication. This chapter has been published in Applied Animal Behaviour Science.

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Chapter 5 is predominantly my own work. I collected video footage of lambs, organised and edited video footage, organised and ran the QBA sessions, undertook the statistical analysis, and drafted the manuscript. DWM assisted in the collection of video footage, helped coordinate fieldwork and the QBA sessions, assisted with statistical analysis and helped review and edit the manuscript. PAF assisted in statistical analysis and in reviewing and editing the manuscript. FA, ALB and SLW assisted in reviewing and editing the manuscript. All authors helped conceive the study, contributed to the experimental design, and approved manuscript for publication. This chapter has been published in Animals.

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Chapter 6 is predominantly my own work. I participated in the setup of the apparatus used to collect video footage, helped organise and edit video footage, organised and ran the QBA sessions, assisted in the collection of feed trough attendance data and in the habituation of sheep to the experimental setup, performed the quantitative behavioural scoring, determined flight speed of animals, undertook statistical analysis and drafted the manuscript. DWM assisted in the collection, organisation and editing of video footage, helped coordinate fieldwork and the QBA sessions, aided in both the collection of feed trough attendance data and the habitation of sheep to the experimental setup, helped with the statistical analysis and assisted in the reviewing and editing of the manuscript. AB assisted in the collection of video footage, helped run the QBA sessions, and assisted in the collection of feed trough attendance data and in the habituation of sheep to the experimental setup. SLW assisted in the collection of feed trough attendance data and assisted with both statistical analysis and reviewing and editing of the manuscript. ALB organised and aided in the collection of feed trough attendance data, and in the reviewing and editing of the manuscript. FA performed the locomotive assessment on sheep to confirm lameness and assisted in the reviewing and editing of the manuscript. PAF assisted in statistical analysis and in reviewing and editing the manuscript. All authors helped conceive the study, contributed to the experimental design, and approved manuscript for publication. This chapter has been published in Applied Animal Behaviour Science.

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PUBLICATIONS ARISING FROM THIS THESIS

Journal publications:

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Conference proceedings:

Grant, E.P., Brown, A., Wickham, S.L., Anderson, F., Barnes, A.L., Fleming, P.A., Miller, D.W., 2015. Qualitative behavioural assessment (QBA) can identify states of sheep wellbeing. Proceedings of Sheep CRC Postgraduate Student Conference. Manly, New South Wales.

Grant, E.P., Brown, A., Wickham, S.L., Anderson, F., Barnes, A.L., Fleming, P.A., Miller, D.W., 2016. Qualitative behavioural assessment (QBA) of remotely captured video footage can identify positive and negative welfare states in sheep. Proceedings of the 31st Biennial Conference of the Australian Society of Animal Production (ASAP) 1239. Adelaide, South Australia.

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Grant, E.P., Brown, A., Wickham, S.L., Anderson, F., Barnes, A.L., Fleming, P.A., Miller, D.W., 2017. Video footage captured in a walk-over-weigh (WoW) system can be used to assess welfare state in sheep. Proceedings of the 51st Congress of the International Society for Applied Ethology (ISAE) Conference. Aarhus, Denmark 209. Wageningen Academic Publishers.

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ABBREVIATIONS

ANOSIM	ANALYSIS OF SIMILARITY
ANS	AUTONOMIC NERVOUS SYSTEM
AWIN	ANIMAL WELFARE INDICATORS
BCS	BODY CONDITION SCORE
EPG	EGGS PER GRAM
FAMACHA	FAFFA MALAN CHART
FCP	FREE-CHOICE PROFILING
FCS	FAECAL CONSISTENCY SCORES
FEC	FAECAL EGG COUNTS
FL	FIXED LIST
GPA	GENERALISED PROCRUSTES ANALYSIS
GPS	GLOBAL POSITIONING SYSTEM
HPA	HYPOTHALAMUS-PITUITARY-ADRENAL
HR	HEART RATE
HRV	HEART RATE VARIABILITY
IRT	INFRA-RED IMAGING TECHNOLOGY
NSAIDS	NON-STEROIDAL ANTI-INFLAMMATORY DRUGS
PCA	PRINCIPLE COMPONENT ANALYSIS

ABBREVIATIONS

PL	PROXIMITY LOGGERS
QBA	QUALITATIVE BEHAVIOURAL ASSESSMENT
RFID	RADIO-FREQUENCY IDENTIFICATION
RR	RESPIRATION RATE
SIMPER	SIMILARITY PERCENTAGE ANALYSIS
SNS	SYMPATHETIC NERVOUS SYSTEM
VAS	VISUAL ANALOGUE SCALES
WOW	WALK-OVER-WEIGH

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CHAPTER 1 LITERATURE REVIEW

1.1 INTRODUCTION

This literature review will introduce and cover the basic concepts of animal welfare and its importance to the sheep industry, summarising and evaluating current and potential tools to measure welfare in terms of validity and on-farm feasibility within the sheep industry. It will focus primarily on behavioural measures and explore the Qualitative Behavioural Assessment (QBA) methodology for its potential in improving the on-farm assessment and monitoring of animals and thus welfare within the Australian sheep industry.

1.2 THE CONCEPT OF ANIMAL WELFARE IN ANIMAL PRODUCTION

Animal welfare is a complex, multi-dimensional concept and despite being the focus of tremendous scientific enquiry on a global scale, there is no single universally endorsed definition. In fact, since animal welfare emerged as a formal discipline following the publication of the Brambell report (Brambell Report, 1965), the concept and definition have evolved over time, thus numerous definitions can be found within the literature (see Table 1.1; and for more detail; Stafleu et al., 1996; Fraser et al., 1997; Fraser, 2008; Haynes, 2008; Broom, 2011; Broom and Johnson, 2019c). Considerable debate concerning what animal welfare represents remains evident within the scientific community, and to give a clear and precise definition of animal welfare has proven difficult. Therefore, in order to assess animal welfare, three main conceptual approaches have emerged to define it namely: i) biological functioning; ii) natural living, and iii) affective state. Although these approaches have been described at length elsewhere (Duncan and Fraser, 1997; Fraser et al., 1997; Fraser, 2003; Szucs et al., 2006; see; Carenzi and Verga, 2009; Fraser, 2009b; Hemsworth and Coleman, 2011; Hemsworth et al., 2015), they are considered here briefly since each contributes to the currently accepted concept of animal welfare.

Table 1.1. Examples of early definitions of animal welfare in the literature.

Definition	Reference
Welfare is a wide term that embraces both the physical and mental well-being of the animal.	(Brambell Report, 1965)
The state of welfare is determined by whether the animal is hungry, thirsty, sexually frustrated, bored, physically uncomfortable, and many other experiences, each of which are generated by processes which have independent biological functions.	(Baxter, 1983)
The welfare of an agricultural animal might theoretically require fulfilment of all of the animal's physiological, safety, and behavioral needs (and some of its wants) most of the time.	(Curtis, 1985)
The welfare of an animal is its state as regards its attempts to cope with its environment.	(Broom, 1986)
A state or condition of physical and psychological harmony between the organism and its surroundings.	(Hurnik, 1988)
To be concerned about animal welfare is to be concerned with the subjective feelings of animals, particularly the unpleasant subjective feeling of suffering and pain	(Dawkins, 1988)
Welfare is to do with what animals feel	(Duncan, 1993)
An animal is in a poor state of welfare only when physiological systems are disturbed to the point that survival or reproduction are impaired.	(McGlone, 1993)
Welfare is the animal's quality of life as it is experienced by and valued by the animal itself.	(Bracke et al., 1999)

1.2.1 Biological functioning

This first approach defines welfare in terms of how well the biological systems of an animal helps it cope with its environment, where good welfare occurs when coping is successful, and failure to cope results in welfare problems (Broom, 1986; Broom, 1991a; Duncan and Fraser, 1997; Hemsworth and Coleman, 2011). In this approach, behavioural and physiological responses are adaptive responses to help the animals maintain homeostasis when faced by challenges, and it is reasoned that both the magnitude of these responses and the biological cost of these responses in terms of health, growth and reproduction, indicate whether the animal is having difficulty coping (Broom, 1991a; Duncan and Fraser, 1997; Hemsworth and Coleman, 2011; Hemsworth et al., 2015). This approach has been widely adopted for many reasons, among which the ease of scientific demonstration of changes in biological functions, and that measures of biological functioning in animal welfare both ensures objectivity and are directly relevant to production industries, are most notable (Duncan and Fraser, 1997; Duncan, 2004; 2005; Sejian et al., 2011). However, criticisms relating to the point at which behavioural and/or physiological change in an animal indicates compromised welfare, and how to separate such changes from routine biological adjustments are also evident (Barnett and Hemsworth, 1990; Duncan and Fraser, 1997; Fraser et al., 1997). Moreover, it is difficult to draw conclusions about an animal's overall welfare state since measures of biological functioning (physiological and behavioural) can be difficult to interpret and may present with conflicting results (Barnett and Hemsworth, 1990; Mason and Mendl, 1993; Duncan and Fraser, 1997; Rushen, 2000; Bracke, 2007). For example, the presence of humans in an open-field test, a fear inducing situation, could induce two different behavioural responses in sheep; immobility or locomotion (Vandenheede et al., 1998). Likewise, rises in cortisol levels are reported after social mixing in lambs (Miranda-de la Lama et al., 2012), but also in lambs in response to play (Chapagain et al., 2014).

1.2.2 Naturalistic behaviour

The naturalistic approach calls for animals to be raised in a 'natural' environment and be managed in such a way that allows the expression of natural behaviours (Duncan and Fraser, 1997; Hemsworth and Coleman, 2011; Yeates, 2018). This approach emphasises that welfare is improved under situations where animals can express their natural behaviours (Hemsworth et al., 2015). This approach is perhaps the least supported by the scientific community, often criticised for its lack of a clear definition of 'natural' and of what constitutes good and poor welfare (Dawkins, 1980; Duncan and Fraser, 1997; Dawkins, 1998; Barnett and Hemsworth, 2009; Hemsworth and Coleman, 2011). Despite this, the concept of providing captive animals with the opportunity to express natural behaviours to potentially improve welfare is an important one and should be considered. Indeed, ideas of 'natural' or 'normal' behaviour are evident in the literature, widely accepted in the public domain, legislation, and are even evident in welfare assessment frameworks such as the Five Freedoms (Freedom # 5 - Freedom to express normal behaviours; Farm Animal Welfare Council, 2009).

1.2.3 Affective state or subjective experiences

The subjective experiences approach emphasises psychological state as an important aspect of animal welfare, where welfare is defined in terms of the subjective experiences (hereafter 'affective state'); the emotions, feelings, and mood, of the animal (Duncan and Fraser, 1997; Hemsworth and Coleman, 2011). Traditionally, under this approach, it was generally considered that the mitigation of negative experiences would improve welfare. This view has been criticised for being too narrow; for not adequately considering the role of positive emotions and experiences to welfare, and it has since been recognised that the promotion of positive experiences and states is a key component of welfare (Boissy et al., 2007; Yeates and Main, 2008; Mellor, 2012; Lawrence et al., 2019). Thus, good welfare occurs when an animal experiences fewer negative emotions (e.g. pain, fear, frustration, boredom), and it experiences positive emotions (e.g. pleasure, happiness, contentedness, curiosity), or at least when the pleasant experiences outweigh the unpleasant ones (Duncan and Fraser, 1997; Hemsworth et al., 2015).

Affective state is an undoubtedly complex and dynamic construct, however, as emotions and moods fluctuate over time and can occur spontaneously, being either short-lived (emotions) or of a longer duration (moods), and are either related to the animal's perception of its external environment or its internal functional state (Mellor, 2012; 2015; Kremer et al., 2020; Paul et al., 2020). Not only is the idea that animals experience emotions controversial, but the fact that it is difficult, if not impossible, to directly measure an animal's affective state, together with issues of objectivity and anthropomorphism, means that this approach is the focus of considerable debate and criticism (Dawkins, 1980; Duncan and Fraser, 1997; Wemelsfelder, 1997; Duncan, 2004; Fraser, 2009a; Proctor, 2012). Regardless of the difficulties in measuring affective state, attempts to evaluate welfare should incorporate affective state (Brambell Report, 1965; Dawkins, 1980; Duncan and Dawkins, 1983; Broom, 1991b; 1996; Duncan, 2004; 2005). After all, we cannot disregard the psychological state of an animal simply because it is difficult to measure and is unconventional, particularly given its relevance to welfare.

1.2.4 The current approach to defining animal welfare

Animal welfare in the twenty-first century is underlined by aspects of the three conceptual approaches, where welfare is currently defined in terms of the ability of an animal to cope with its environment, both physically (fitness and health) and psychologically (emotionally) (Duncan and Fraser, 1997; Webster, 2005a; Boissy et al., 2007; Hemsworth et al., 2015). Under this framework, good welfare occurs when an animal is in good physical condition, is healthy and functioning properly (e.g. growth, fertility), and is predominantly free from unpleasant affective states (e.g. pain, fear) yet experiences pleasant emotions (e.g. happiness, pleasure). The key elements of animal welfare, as we presently understand it, are listed as follows:

- Welfare is a state within the animal itself – a characteristic per se (Broom, 1991a).
- Welfare is dynamic and may vary along on a continuum between very bad to very good (Broom, 1991a).
- Welfare relates to both the physical fitness and health, and the psychological well-being, or affective state, of an animal (Duncan and Fraser, 1997; Webster, 2005a; Boissy et al., 2007; Hemsworth et al., 2015).
- Animals should have a 'good' life not just a 'not-so-bad' life – good welfare involves the promotion of positive states not just the reduction of negative ones (Boissy et al., 2007; Yeates and Main, 2008).
- The range of affective states of importance (both positive and negative) has been expanded from the original 'Five Domains' (freedom from thirst, hunger, anxiety, fear, pain/distress) (Mellor and Beausoleil, 2015) and the additional importance of pleasure states has been recognised (Balcombe, 2009).

1.3 IMPORTANCE OF ANIMAL WELFARE IN THE SHEEP INDUSTRY

1.3.1 Why is animal welfare important?

As in other animal production industries, there is no doubt that animal welfare is important to the sheep industry. The four key reasons that underpin the importance of welfare to the sheep industry; i) ethical and moral reasons, ii) social licence, iii) legislation, and iv) production, will be considered here.

1.3.1.1 Moral and ethical implications of the domestication of animals for production

Foremost, animal welfare should be considered a priority since species domesticated for production purposes are dependent on humans for their care and ultimately survival (Benson et al., 2004; Hemsworth and Coleman, 2011). Domestication is the process by which captive animals adapt to man and the environment provided (Price, 1984), and the confinement of animals within man-made environments may restrict the opportunities for the animal to meet vital behavioural and physical needs. An animal's capacity to perform highly motivated behaviours is commonly restricted under commercial systems, and such restrictions are considered to compromise welfare (Dawkins, 1980; Dawkins, 1988; Broom and Johnson, 1993). Furthermore, while considered necessary for health or management purposes, production animals are often exposed to routine or sporadic procedures and practices that may compromise welfare (Nordquist et al., 2017). Indeed, common practices such as castration and shearing in sheep are known to be stressful and/or painful (e.g. Hargreaves and Hutson, 1990a; Molony et al., 1993). Essentially, producers control the experiences, both positive and negative, that animals are exposed to during their lifetime, and as the primary caretakers of these animals, they have a moral and ethical responsibility to ensure animals receive the provisions necessary for survival (e.g. food, water, shelter, and veterinary care), and to safeguard the welfare of the animals in their care.

From an ethical standpoint, the views, positions, and attitudes on moral obligations to, and the protection of, animals under human care arise from many perspectives, namely, i) deontology, ii) utilitarianism, iii) contractarian, and iv) respect for nature. The first two are the prominent, conflicting, positions and as such will be briefly discussed here. The deontological, or animal rights, perspective views animal welfare in terms of fixed ethical rules, recognising that every animal has equal worth and rights, and these should be protected (Regan, 1983). Briefly, it is the morality of the action, not the result of the action that is considered, meaning that from a pure deontological view, animals should not be exploited for human purposes (Regan, 1983). Whereas the utilitarian, or animal welfare, perspective considers the strength and nature of the impacts for both animals and humans, to evaluate the overall impact (Singer, 1975). As such, activities that have some consequences for animal welfare could be considered acceptable under this view if the benefits to animals and/or humans outweigh the consequences i.e. net increase in welfare (Singer, 1975). These views, in their 'pure' forms, do not address the complexity of the issues of animal protection, but these perspectives persist in less radical, hybrid forms and underpin understanding of morality with regards to the use of animals for human purposes (Broom, 2003; Würbel, 2009).

1.3.1.2 Considering animal welfare, the public and the social licence to farm

Good animal welfare has now become an important societal issue, and there is the expectation that animals raised in production industries receive high levels of care and are treated humanely (Blokhuis et al., 2003). The failure to meet these expectations diminishes public acceptance and trust, posing a threat to the 'social licence' to farm (i.e. public acceptance and support), the consequence of which is criticism, and the possible loss of market access (van der Meulen and Freriks, 2006; Arnot, 2008; Martin and Shepheard, 2011). Furthermore, societal opposition can force the government to intervene to protect and improve welfare on farms (Miele et al., 2013). The main means through which governments achieve this is the introduction of new or amendments to legislation to tighten regulation or litigation to protect welfare (van der Meulen and Freriks, 2006; Arnot, 2008; Martin and Shepheard, 2011). So, to remain competitive and to maintain their social licence, animal production industries must project a welfare-friendly image and be seen to operate in alignment with public values.

In Australia, sheep producers and the general public share key attitudes towards sheep welfare, and both consider welfare important (Doughty et al., 2017). However, this has not always been the case, and the criticism of the live export sector and the debate regarding the mulesing of sheep to protect against flystrike are recent examples of instances where social licence was threatened (Ferguson et al., 2014; Coleman, 2017; 2018). In both of those cases, the attitudes and actions of the public brought about ongoing changes to the industry including the increased use of the local anaesthetic Tri-Solfen® during mulesing, the development of alternatives to mulesing, and the review of the Australian Standards for the Export of Livestock. Furthermore, since public attitudes and expectations may change over time (Coleman, 2017) or from the exposure to negative media coverage (Tiplady et al., 2013; Sinclair et al., 2018), the sheep industry may again face opposition from the public and/or animal advocacy groups which may force producers to change or abandon some activities (e.g. mulesing) or cause the loss of market access (e.g. the banning of live export of sheep). These examples serve to highlight the importance of meeting, if not exceeding, societal expectations of animal care, and the consequences of tenuous social license.

1.3.1.3 Legislation and welfare standards

There are several mechanisms in place to protect the welfare of farm animals across developed countries in response to increasing societal concern (see; Barnett and Hemsworth, 2009). In Australia, animal welfare practices are governed by The Australian Animal Welfare Standards and Guidelines, which update and replace the Model Code of Practice for the Welfare of Animals for various industries, including the sheep industry. These detail standards of welfare that must be met under the law and are accompanied by voluntary guidelines for recommended practices for the care and husbandry of animals (sheep; Animal Health Australia, 2014a; and cattle; Animal Health Australia, 2014b). Under the Australian Animal Welfare Strategy (AAWS, 2005) these standards are currently being integrated into legislation across the States and Territories and ensure minimum welfare standards are upheld. For sheep, these standards detail important matters including requirements of feed and water, risk management of disease, injury and predation, handling, tail docking and castration, mulesing, breeding management and humane killing (Animal Health Australia, 2014a). One standard of particular importance states that; *"a person in charge must ensure the inspection of sheep at intervals, and at a level appropriate to the production system and the risks to the welfare of sheep"* (Animal Health Australia, 2014a). Therefore, it is not only in the best interest to producers to monitor the welfare of their animals, but also a requirement. Consequently, there is a strong imperative in the industry for the development of methods to monitor animal welfare on-farm, not only to improve welfare but to demonstrate compliance with the standards of welfare as detailed in the legislation.

1.3.1.4 Production and profitability

The final, but perhaps most compelling reason underpinning the importance of welfare to the sheep industry is production and profitability. Although the relationship between poor welfare and production is complex, there is considerable evidence that animals with poor welfare produce less and are in poor health (Cottle, 1991; Miele et al., 2013), and this pattern is clearly evident in sheep (see Table 1.2). Internal parasites, for instance, are estimated to cost approximately $310 million in reduced income to Australian sheep producers annually, whereas flystrike (body strike, breech strike and pizzle strike) and lice cost $82.7 million and $38.9 million, respectively (Sackett et al., 2006). Given that poor welfare jeopardises production and profit, it is not surprising that producers are concerned with animal welfare and are motivated to improve it (Blokhuis et al., 2010; Kauppinen et al., 2010; Blokhuis et al., 2013; Doughty et al., 2017). If poor welfare reduces profits, then it follows that improvements to animal welfare are associated with notable financial benefits. Indeed, the benefits of improved welfare arise from reductions in mortality, improved health, increased product quality and increased resistance to disease, thus reduced treatment costs (Dawkins, 2017), and stakeholders acknowledge that a benefit of good welfare is the improvement in production (Sinclair et al., 2019).

Table 1.2. Common welfare issues in sheep and their effect on production, reproduction and/or health as derived from the literature.

Welfare issue	**Impact on production, reproduction and/or health**									**References**
	Reduced growth rates or weight loss	**Reduced wool growth**	**Reduced wool quality**	**Reduced milk yield**	**Altered milk composition**	**Reduced ewe reproductive efficiency**	**Reduced ram fertility and/or mating success**	**Reduced birthweights, growth rates and/or lamb survival**	**Compromised immunity and/or disease susceptibility**	
Inadequate nutrition	■	■	■	■	■	■	■	■	■	Kellaway (1973); Oldham et al. (1978); Gunn et al. (1984); Jordan and Mayer (1989); Masters et al. (1998); Olivier and Olivier (2005); Tsiplakou et al. (2012); Guan et al. (2014); Grant et al. (2016)
Water deprivation	■			■	■			■	■	Lynch et al. (1972); Barbour et al. (2005); Kumar et al. (2016); Casamassima et al. (2018)
Heat stress	■	■		■	■	■	■	■	■	Dutt et al. (1959); Dutt (1964); Thwaites (1967); Alexander and Williams (1971); Abdalla et al. (1993); Sevi et al. (2001a); Finocchiaro et al. (2005); Abi Saab et al. (2011); Alhidary et al. (2012); Caroprese et al. (2018)
Mastitis				■	■			■		Fthenakis and Jones (1990); Albenzio et al. (2002); Leitner et al. (2004); Griffiths et al. (2019)
Lameness	■	■		■		■	■	■		Stewart et al. (1984); Marshall et al. (1991); Nieuwhof et al. (2008); Gelasakis et al. (2010); Wassink et al. (2010); Tibary et al. (2018)
Parasitism	■	■	■	■		■	■	■	■	Symons et al. (1981); Leyva et al. (1982); Cobon and O'Sullivan (1992); Suarez et al. (2009); Mavrogianni et al. (2014); Fthenakis et al. (2015 and references within); Mavrogianni et al. (2017); Horton et al. (2018)

1.3.2 A brief overview of the major welfare concerns in the Australian sheep industry

"Sheep… lead romantic lives, free from suffering, because they wander freely on hillsides, unconstrained by cages and other inventions of modern farming" (Dawkins, 1980; p. 53).

In Australia, sheep are reared predominantly under extensive systems that are characterised as easy-care and low management input in terms of labour, where animals are kept predominantly outside on pasture. Although it is thought that the welfare of sheep in such systems is improved by their more 'natural' life (Sevi et al., 2007; Dwyer, 2009), they are nevertheless vulnerable to several challenges that may compromise their welfare. These challenges come in the form of climate variability, inadequate resource provision, predation, and untreated disease and/or injury (see; Goddard et al., 2006; Sevi et al., 2007; Dwyer, 2009), and many of these challenges require prompt recognition and action to avoid compromises to production, health, and welfare. From a welfare standpoint, the lack of close daily contact between producers and animals, outside of key production times (e.g. lambing, marking, weaning, or shearing), is important since it means that individuals that are in need of treatment may go unnoticed for extended periods or may even be missed (Goddard et al., 2006). For example, Munoz et al. (2019) found that of 6,200 ewes assessed across 32 farms between mid-pregnancy and weaning, 185 (3%) needed care, considered to have compromised welfare from issues including lameness and active dermatophilosis (broken wool).

In Australia, and indeed globally, health status is one of the major welfare concerns in sheep. Major diseases of importance in Australia include foot-rot, internal and external parasites, and ovine Johne's disease, where plant poisoning is also considered an important condition (Plant, 2007). Animals in poor health have reduced physical health, disease resistance, fertility and production, and producers incur large financial costs from such losses, in addition to those costs arising from treatment (Sackett et al., 2006; Dwyer and Lawrence, 2008; Roger, 2008; Lane et al., 2015). Alongside health status, other important welfare issues in the Australian sheep industry include the provision of adequate nutrition, physical injury, distress or chronic stress, painful or distressing husbandry practices (e.g. mulesing and tail docking) and poor human-animal relationships or interactions (Coop and Kyriazakis, 1999; Hemsworth, 2003; Dwyer and Bornett, 2004; Grant, 2004; Winter, 2004; Athanasiadou et al., 2008; Dwyer, 2009; Munoz et al., 2018; Munoz et al., 2019). There are clear welfare issues in the Australian sheep industry and producers face many challenges when caring for and managing sheep, for which they need tools and training to allow them to easily and promptly recognise and manage animals in need.

1.4 THE ASSESSMENT OF ANIMAL WELFARE: POSSIBLE TOOLS FOR THE ASSESSMENT AND MONITORING OF SHEEP

Producers routinely use prophylactic (disease prevention) strategies to minimise risks to health and welfare, but they also need to monitor environmental conditions to determine whether animals should be managed differently or if stock need to be treated strategically to prevent disease if conditions change (for example, if animals need flystrike-prevention treatment during hot/wet conditions). Furthermore, the monitoring of animals is fundamental in efforts to improve welfare in commercial situations. Without protocols to identify animals in compromised welfare states, we may miss the opportunity to treat an animal early and ease its suffering or, worse, overlook the issue until it is too late, when mortality is certain. Not only do producers need to be able to reliably and easily identify individual animals in states of compromised welfare from large-sized flocks, they also must make appropriate decisions concerning time, type, and extent of intervention. Furthermore, to meet welfare standards and societal expectations, producers are required to monitor their stock to identify those in need of treatment. For these reasons, there is a strong imperative in the industry for the development of simple, cost-effective methods to monitor sheep on-farm.

To develop methods to monitor sheep, it is important to consider briefly what is needed to assess welfare under extensive systems, or indeed all commercial systems. Several reviews are available covering various aspects of on-farm welfare assessments and evaluating various welfare measures (e.g. Barnett and Hemsworth, 1990; Main et al., 2003; Webster et al., 2004; Webster, 2005b; Edwards, 2007; Barnett and Hemsworth, 2009; Knierim and Winckler, 2009; Main et al., 2014). It is evident from such reviews that there are several fundamental attributes necessary for welfare measures and assessments under commercial farm conditions:

- Transparent and uncomplicated; clearly and fully understood by stock persons.
- Comprehensive; a combination of resources- and animal-based measures that addresses each aspect of welfare.
- Simple to use and time-effective (feasible), but informative and robust (valid, repeatable, and meaningful).
- Flexible to adapt to different production systems or changes on-farm.

The diversity and breadth of welfare measures available for the purpose of assessing animal welfare are immense; however, it is beyond the scope of this review to discuss all in detail. Instead, key measures that have emerged to assess the welfare state of individual animals from two main categories will be examined, namely, i) resource-based; and ii) animal-based.

1.4.1 Resource-based measures

The provision of adequate resources is fundamental for ensuring good health and welfare of stock (Appleby and Waran, 1997) and various measures have been developed that quantify, monitor and assess such resources. Key resource measures include both water and food availability and accessibility, space allowances, stocking density, group size and composition, access to shelter, temperature, ventilation, and design of facilities (Blokhuis et al., 2003; Webster, 2005a; Barnett and Hemsworth, 2009; Goddard, 2011; Main et al., 2014). It is noteworthy that such measures are not limited to the physical environment but can include parameters relating to management such as competent stockpersonship and appropriate husbandry, including the skilled recognition of signs of illness and injury, appropriate preventative actions and treatment of ill or injured animals (Main et al., 2001; Main et al., 2003; Webster, 2005a; Webster, 2005b). Essentially, these measures answer the question of whether the animals have what they need to live a healthy life and for potential risks to welfare to be identified.

In sheep, Phythian et al. (2011) identified 35 management- or resource-based measures useful in the assessment of the welfare of sheep on-farm, and various studies have demonstrated the usefulness of resource-based measures in practical assessments (e.g. Napolitano et al., 2009; Stubsjøen et al., 2011). Many of the existing assurance or accreditation schemes are primarily based on resource-based measures (Main et al., 2001; Webster et al., 2004; Webster, 2005b). Such measures have been widely adopted not simply because they are amongst the easiest to collect under commercial conditions (Rushen et al., 2011), but because they are argued to be more reliable than subjective, animal-based measures (Main et al., 2001). However, these measures do not assess the animal per se (indirect), and thus are not true measures of welfare (Main et al., 2001; Webster et al., 2004). Rather resource- and management-based measures can be viewed as input factors, measures that provide valuable information to determine risk of poor welfare and aiding in the interpretation of output (animal-based) measures of welfare (EFSA, 2012). In this way, such measures are important and complementary to other measures. It should also be emphasised that fulfilling the basic needs of an animal does not necessarily guarantee good welfare (Webster, 2005b). For these reasons, there is the obvious need for welfare assessments to go beyond simply monitoring resources. Nevertheless, these measures are important to inform sheep welfare (Goddard, 2011), and monitoring of resources allows consideration of whether the basic physical needs of the animals are met, and are valuable from a risk assessment standpoint.

1.4.2 Animal-based measures

In the assessment of animal welfare, animal-based measures which measure the animal itself (i.e. output-based) are considered most informative. A wide and diverse range of animal-based measures exist that may inform sheep welfare. Broadly these fit into three categories: i) physical condition and production performance; ii) physiological, and iii) behavioural. It is important to note that in using animal-based measures we do not measure welfare state per se, rather we measure the changes that challenge cause in the animal, and the responses the animal uses in its attempts to cope with these challenges (Etim et al., 2013). In this way, measures of health and production reflect physical changes in animals in response to difficulty coping, while measures of physiology and behaviour reflect the adaptive responses the animal employs to help cope with its environment. A discussion of the staggering number of animal-based measures to assess sheep welfare would make this review impossibly long, and many have already been reviewed (e.g. Dwyer and Bornett, 2004; Lovatt, 2010; Llonch et al., 2015; Richmond et al., 2017; Barrell, 2019; Mattiello et al., 2019), thus only key measures from each of the preceding categories that have the potential to meaningfully inform the welfare of sheep under commercial conditions will be considered. It will draw heavily from laboratory and experimental literature because most outcome-based measures of welfare arise from these situations and those formal protocols or schemes tend to focus on resource availability (i.e. input-based). Given the focus of this thesis, where possible, specific reference will be made to work on sheep.

1.4.2.1 Measures of physical health, physical condition, production, and performance

Measures of physical condition and production and performance are useful to inform welfare, indicating when an animal has failed or is failing to cope with a challenge (Broom, 1986; Broom and Johnson, 1993; Webster, 2005a). Examples of key physical health and condition measures used in sheep welfare assessments are presented in Table 1.3. These have been widely adopted and many feature in formal protocols such as the Animal Welfare Indicators Project (AWIN) for Sheep (AWIN, 2015d), or in guides designed to help producers manage welfare such as the Visual Scores Guide in sheep to manage flystrike (AWI, 2007) and the Five Point Check© for the targeted selection of animals for treatment of internal parasites (Bath and van Wyk, 2009). In particular, the measurement of body condition score (BCS) is valuable since changes can be linked to malnutrition, parasitism and disease (e.g. van Burgel et al., 2011; Cornelius et al., 2014; Smith et al., 2014; Horton et al., 2018), and can be used to predict ewe survival in extensive systems (Morgan-Davies et al., 2008).

In terms of performance, measures of reproductive success (lambing and weaning rates), production (milk or wool yield and quality) and mortality (culling rate and life expectancy) are commonly assessed given the link between welfare and performance (see; 1.3.1.4 Production and profitability; Table 1.2). Physical and performance measures can be valuable in welfare assessments; however, they are limited in that they do not address the psychological aspect of welfare, and that good physical condition and production performance do not necessarily equate to good welfare (Dawkins, 1980). Physical and performance measures are also limited in that they are often retrospective in nature; 'lag indicators' (Barnett and Hemsworth, 2009), used not to help current animals, but future ones. As such, there is the need for 'lead indicators' that inform producers of an issue early to allow for corrective and preventative actions to be taken to help the current animals (Barnett and Hemsworth, 2009). Furthermore, caution is warranted when interpreting these measures in welfare terms since they may be affected by a number of factors other than welfare such as diet and activity level (e.g. Mason and Mendl, 1993). Lastly, since they generally require the gathering and handling of stock (Weary et al., 2006), management restrictions (labour, time and resources) are likely to limit the number of different measures producers can perform under commercial conditions.

Table 1.3. Examples of key physical health and condition indicators in sheep to inform welfare.

Measure	Reference
Skin condition (e.g. irritation, presence of lesions)	Caroprese et al. (2008); Lovatt (2010); Phythian et al. (2016); Munoz et al. (2018); Munoz et al. (2019)
Fleece condition (e.g. colour, break, fleece rot)	Phythian et al. (2016); Munoz et al. (2018); Munoz et al. (2019)
Cleanliness	Napolitano et al. (2009); Mialon et al. (2011); Phythian et al. (2016); Phythian et al. (2019)
Dag score	Broughan and Wall (2007); Greeff et al. (2014); Munoz et al. (2019)
Urine stain score	Greeff et al. (2014); Greeff et al. (2018)
Mucosa colour	Bath and Van Wyk (2001); Malan et al. (2001); Van Wyk and Bath (2002); Kaplan et al. (2004)
Eye condition (e.g. eye abnormalities, ocular discharge, lesions)	Phythian et al. (2013b)
Body condition (e.g. body condition score; BCS)	Russel (1984); Morgan-Davies et al. (2008); Napolitano et al. (2009); (Stubsjøen et al., 2011); Phythian et al. (2013b); Horton et al. (2018)
Disease incidence (e.g. lameness, mastitis, and parasites)	Kaler and Green (2008); Cringoli et al. (2009); Napolitano et al. (2009); Phythian et al. (2016); Munoz et al. (2018); Phythian et al. (2019)

1.4.2.2 Physiological measures

Challenge evokes a series of physiological responses in animals, designed to remove the challenge or to help the animal minimise or 'cope' with its impact (Webster, 2005a). These responses include the activation of the hypothalamus-pituitary-adrenal (HPA) axis, autonomic nervous (ANS) and immune systems, and measures of these systems can inform welfare, indicating when an animal is being challenged. Although, in practice, physiological measures do not usually contribute to on-farm assessment schemes, they will be considered in brief here since they can provide meaningful information relevant to welfare, are important for validation purposes, and since technological advancements could improve the feasibility of some measures for on-farm application.

1.4.2.2.1 Hypothalamic-pituitary-adrenal (HPA) activity

The most-used physiological response employed to evaluate stress and welfare in animals is the activation of the HPA axis. The role of HPA axis and glucocorticoids (cortisol) in the stress response have been extensively detailed elsewhere (see; Matteri et al., 2000; Sapolsky et al., 2000; Mormède et al., 2007; Kuo et al., 2015; Ralph and Tilbrook, 2016b; Broom and Johnson, 2019b); here I offer a brief, although perhaps simplistic, summary. To respond to a stressor (external or internal, physical or psychological), animals must expend energy (e.g. increased locomotor activity, increased alertness or defence), and the HPA axis and its end-product, glucocorticoids (hereby cortisol), are one of the important mechanisms by which a stressed animal mobilises that energy (Sapolsky et al., 2000; Parker, 2003; Reeder and Kramer, 2005; Kuo et al., 2015). Circulating cortisol concentrations have been the primary physiological means to evaluate stress in animals. Elevated concentrations of circulating adrenocorticotropic hormone, β-endorphin, and vasopressin, along with measures of plasma metabolite concentrations (e.g. glucose levels) may also be used to indicate activation of the HPA axis with varied success and/or ease (Reeder and Kramer, 2005; Webster, 2005a; Mormède et al., 2007; Barrell, 2019).

In sheep, cortisol has been investigated under numerous physically and/or psychologically challenging situations (Table 1.4) and has been proposed as a useful measure of stress and thus welfare. Indeed, elevated cortisol concentrations are easy to obtain (Webster, 2005a) and are regarded as a sort of ‘silver bullet’ for indicating physiological stress in animals (Barrell, 2019). However, the use of elevated cortisol concentration to indicate stress is now regarded with caution and several major limitations are widely recognised. In fact, Ralph and Tilbrook (2016b) and numerous others (e. g. Barnett and Hemsworth, 1990; Mormède et al., 2007; Hart, 2012; Cockrem, 2013; Otovic, 2014) have cautioned the use of cortisol in assessments of welfare over the years, highlighting several considerations alongside practical issues. These considerations are: (i) difficulty in identifying original stimulus; (ii) variability caused by circadian rhythms; (iii) the invasive and labour-intensive nature of the sampling procedure; (iv) the need for multiple samples with specific storage requirements (e.g. refrigeration, freezing and separation via centrifuge); and (v) individual variation (e.g. age- and sex- and previous experience-related variation).

Table 1.4. Examples of physically and/or psychologically challenging situations that evoke a cortisol response in sheep.

Challenge	References
Shearing	Hargreaves and Hutson (1990b; 1990a); Mears et al. (1999)
Weaning	Orgeur et al. (1999); Godfrey et al. (2016)
Presence of predators	Harlow et al. (1987); Ralph and Tilbrook (2016a)
Social isolation	Parrott et al. (1988); Parrott et al. (1994); Degabriele and Fell (2001); Caroprese et al. (2010)
Physical restraint	Minton et al. (1992); Niezgoda et al. (1993); Mears and Brown (1997)
Handling	Hargreaves and Hutson (1990b); Mears et al. (1999); Yardimci et al. (2013)
Social mixing	Hall et al. (1998); Miranda-de la Lama et al. (2012)
Transport	Bradshaw et al. (1996); Hall et al. (1998); Smith and Dobson (2002)
Painful husbandry procedures e.g. castration and mulesing	Fell and Shutt (1989); Mellor and Murray (1989); Chapman et al. (1994); Lester et al. (1996); Thornton and Waterman-Pearson (1999); Hemsworth et al. (2009).

From a welfare assessment perspective, it is also an important realisation that both positive and negative affective states evoke similar HPA activity (Ralph and Tilbrook, 2016b). For example, elevated cortisol concentrations are reported to be associated with mating in cattle, pigs and horses (Borg et al., 1991; Villani et al., 2006), exercise in sheep (Apple et al., 1994; Cockram et al., 2012), feeding or food anticipation in pregnant ewes and cattle (Willett and Erb, 1972; Simonetta et al., 1991) and play in lambs (Chapagain et al., 2014). Consequently, interpretation is not always straightforward and cortisol values offer little to the understanding and assessment of the psychological aspect of welfare.

To overcome some of the practical limitations for blood sampling, several studies on ruminants have investigated cortisol (or its metabolites) in biological matrices such as milk (Verkerk et al., 1998; Sgorlon et al., 2015), saliva (Fell et al., 1985; Fell and Shutt, 1986; Negrão et al., 2004), faeces (Palme et al., 1999; Palme et al., 2000) and wool (Caroprese et al., 2010; Ghassemi Nejad et al., 2014; Fürtbauer et al., 2019). While all of these have advantages over blood cortisol (e.g. ease of sampling and storage and the non-invasive manner of collection, see; Russell et al., 2012; Heimbürge et al., 2019), they too have limitations. For example, while wool cortisol can indicate long term stress in sheep (e.g. Stubsjøen et al., 2018; Sawyer et al., 2021; Weaver et al., 2021), to assess wool cortisol multiple samples are required, which must be collected from the same body location (Fürtbauer et al., 2019; Heimbürge et al., 2020). Furthermore, as recognised by Stubsjøen et al. (2018), levels in wool may reflect not only systemic glucocorticoid levels but also local production in the wool follicle making this measure difficult to interpret.

1.4.2.2.2 Autonomic Nervous System (ANS) activity

The ANS, specifically the sympathetic nervous system (SNS), is activated during the stress response to prepare the animal to mount a rapid response to the challenge and restore homeostasis, commonly referred to as the ‘fight or flight’ response. Both the HPA and SNS systems are integral to the mobilisation of energy during the stress response and, once activated, work in synergy, offering a coordinated response that ultimately increases circulating glucose concentrations (Sapolsky et al., 2000; Reeder and Kramer, 2005). Briefly, activation of the SNS causes the secretion of catecholamines; noradrenaline and adrenaline (also known as norepinephrine and epinephrine) which increase arousal, elevates heart rate, cause vasoconstriction that elevates blood pressure, and mobilise energy stores via glycogenolysis and lipolysis (Matteri et al., 2000; Parker, 2003; Reeder and Kramer, 2005). Thus, measures of either the hormonal response evoked during activation or the biological endpoint of SNS activation may provide information useful for the assessment of welfare.

To assess SNS activation, firstly, plasma levels of catecholamines may be used. The measurement of catecholamines have been used relatively infrequently to study stress in livestock; however, a few studies in sheep have included measures of catecholamines to gain a more comprehensive physiological assessment of; (i) castration (Mellor et al., 2002), and (ii) psychological (isolation) and physical (transport simulation, standing in water and control handling) challenges (Parrott et al., 1994). The monitoring of SNS activity via hormone measures is difficult because they are only available for collection for brief periods of time, either rapidly removed or metabolised soon after release (Reeder and Kramer, 2005; Webster, 2005a). Furthermore, as with the use of cortisol to indicate HPA axis activation, it is likely that sampling for catecholamines would be an issue in terms of practicality, invasiveness, and cost. Notably, positive (not just negative stimuli) can evoke the synthesis of catecholamine (Ralph and Tilbrook, 2016b). In addition, the significance of elevated catecholamines in relation to sheep welfare is not well known, and for these reasons, adoption is unlikely.

The second means to assess SNS activity is through the biological effects of activation, including heart rate (HR), body temperature (T_b) and respiration rate (RR) (Reeder and Kramer, 2005). Although these measures offer only an indirect indication of SNS activity, they are more accessible for assessment. They also have merit in that their assessment is (generally) non-invasive in nature (Webster, 2005a), although they can still be intrusive and may require the use of expensive equipment (e.g. heart rate monitors). These measures are commonly used to assess sheep in a variety of stress and welfare studies, although RR is perhaps the least well developed as an indicator of SNS stress, as it is commonly only used to assess heat and cold stress (Webster, 2005a), and will not be discussed further here.

The use of cardiac variables (i.e. HR), heart rate variability (HRV) and its indices, in particular, are common across livestock species (reviewed by; von Borell et al., 2007). In sheep they have been used, for example, to assess the impact of shearing (e.g. Hargreaves and Hutson, 1990a), predator presence (e.g. Roussel et al., 2004), human interactions (e.g. Coulon et al., 2015), transport (e.g. Wickham et al., 2012), pain (e.g. Stubsjoen et al., 2009), social isolation and handling (e.g. Baldock and Sibly, 1990), the response to sudden, unpredictable and novel situations (Desire et al., 2004) and controllable and uncontrollable events (Greiveldinger et al., 2009). HR and HRV reflect SNS activation and provide a measure of the balance of activity between the sympathetic and parasympathetic (or vagal) divisions of the ANS (von Borell et al., 2007; Barrell, 2019). However, since behaviour (von Borell et al., 2007) together with age, sex and past experiences (Bohus et al., 1987), strongly affects cardiac activity, problems can arise when interpreting changes in HR and HRV. Furthermore, for practical and experimental application, the monitors or bio-loggers themselves must not affect the animal (Broom and Johnson, 1993). As such, it must be considered whether animals require 'training' or a period of acclimation to such monitors, which may complicate feasibility under practical conditions.

Body temperature is also an indirect measure of SNS activation and may be useful to inform welfare assessment (Reeder and Kramer, 2005; Webster, 2005a). T_b measurements can be made using internal loggers (rectal, vaginal, ruminal, sub-cutaneous) or more recently, through infra-red imaging technology (IRT) (reviewed by; Sellier et al., 2014). Various studies have demonstrated the usefulness of T_b measures collected through various means to assess acute physical and psychological stress in sheep (e.g. Parrott et al., 1999; Pedernera-Romano et al., 2010; Cannas et al., 2018). Of particular benefit for the assessment of welfare is that measures of body surface temperature and humidity (via biosensors attached to the skin) alongside cardiac measures have been reported useful to differentiate emotional valence (positive or negative) in sheep (e.g. Reefmann et al., 2009c). The use of biosensors to measure T_b are costly and are invasive or intrusive, and are therefore unlikely to be useful for practical application (Richmond et al., 2017). However, the advancement of IRT has facilitated the non-invasive assessment of T_b, improving the usefulness and feasibility of T_b measures under practical conditions. For example, George et al. (2014) found that the temperature of the eye can be used as an alternative to invasive vaginal or rectal temperature to indicate T_{core} in sheep. However, T_b is influenced by increased activity, diet, and environmental factors in addition to being subject to diurnal rhythms, and there are also problems surrounding the standardisation of camera and temperature sensing device position (Sellier et al., 2014; Barrell, 2019).

Importantly, measures of T_b are also useful in the detection of disease and infection in animals, since changes can reflect elevated temperatures caused by fever and/or inflammatory processes (reviewed by; McManus et al., 2016). In sheep, for instance, IRT has been reported useful to detect foot lesions in lame rams (Talukder et al., 2015), classify mastitis in ewes (Martins et al., 2013), to detect inflammation and/or infection caused by ear-tagging (Karakuş and Karakuş, 2017), and to detect fever in sheep experimentally infected with bluetongue virus (Pérez de Diego et al., 2013). Although these changes in T_b are not strictly caused by the activation of the SNS during an acute stress response, the identification of these changes has been presented here because the assessment of disease and infection is of great concern for welfare assessment in sheep (1.3.1.4 Production and profitability). Overall, measures of T_b, particularly non-invasive measures (i.e. IRT) may prove advantageous in practical welfare assessments being capable of indicating both physiological stress and disease or infection.

Eye aperture or visible eye white is another physiological measure of ANS activity that can inform welfare. Although only recently explored in livestock, there is promising evidence that suggests changes in visible eye white could provide information concerning affective state in sheep (Reefmann et al., 2009c; Tamioso et al., 2017; Tamioso et al., 2018). Of particular benefit for the assessment of welfare is that measures of visible eye white may be useful indicators of positive affective state, which are necessary but notably lacking from the welfare monitoring 'toolbox'. For example, it has been shown that ewes experiencing positive contact with humans (brushing) displayed a greater proportion of closed or half-closed eyes during the event than those ewes that were exposed to the human but were not brushed (Tamioso et al., 2018). The majority of research into visible eye white has been done on cattle (e.g. Sandem et al., 2002; Sandem et al., 2004; Proctor and Carder, 2015), however, and the validity and reliability of such measures in sheep remain to be verified. Concerns have also been raised over data collection technique (Reefmann et al., 2009b) and, although measures of eye aperture have an advantage being non-invasive, the labour-intensive nature of recording means that feasibility for on-farm application is low (Mattiello et al., 2019).

1.4.2.2.3 Immune function

Measures of immune function may also provide a means to assess animal welfare. On detection of a pathogen or toxin the body's' immune system launches a sophisticated response designed to defend against and/or rid the body of the infectious agent(s) (Chaplin, 2010; Nicholson, 2016). Evidence of immune response is apparent in sheep suffering from various diseases and infections such as gastrointestinal nematode infection (McRae et al., 2015), ectoparasitic disease (Wells et al., 2013), mastitis (Queiroga, 2018) and paratuberculosis or Johne's disease (Burrells et al., 1998). Tissue damage or injury may also evoke the innate immune response in terms of the acute phase response (Cray et al., 2009; Eckersall and Bell, 2010). For example, surgical pulmonary tissue damage (Pfeffer and Rogers, 1989) and tissue damage caused by mulesing (Lepherd et al., 2011) are associated with increased and/or sustained concentrations of plasma acute phase proteins in sheep suffering from various diseases. Thus, measures of immune response are useful to detect disease and injury which is necessary when determining an animal’s welfare state. Furthermore, measures of immune function can indicate that an animal is having difficulty coping with its environment since animals often show some degree of HPA glucocorticoid-mediated immunosuppression when challenged (Broom and Johnson, 2019b). For example, Caroprese et al. (2010) detailed the immune response of lactating dairy ewes in response to isolation stress in terms of leukocyte population, and blood and whey interleukins, finding changes in the immune profile of the ewes related to HPA axis reactivity.

In terms of the assessment of animal welfare, several measures of immune function have been reported to add value to welfare assessments in that they indicate an activation of the immune system in response to a pathogen or indicate reduced immune function. In sheep, these include immunoglobulins or specific antibody levels in plasma, saliva or colostrum (Barrell, 2019; Broom and Johnson, 2019a), T-cell activity and proportion of T-cell subsets (e.g. cytotoxic T-cells, T-helper cells and total lymphocytes) in the blood (Caroprese et al., 2010; Broom and Johnson, 2019b), acute phase response protein levels (Cray et al., 2009; Lepherd et al., 2011), leukocyte profiles or the neutrophil-to-lymphocyte ratio (Paull et al., 2008; Pascual-Alonso et al., 2017) and somatic cell counts in milk (Sharma et al., 2011). However, most require at the very least the handling and restraint of animals to draw a sample (e.g. blood, saliva, or milk), and some also require more extensive (and expensive) laboratory analysis. For these reasons, they are arguably impractical for commercial application. Furthermore, like measures of neuroendocrine activity, measures of immune function can have issues with interpretation since there are individual (Terlouw et al., 1997) and breed-specific differences in immune response (e.g. Hadfield et al., 2018), and responses are influenced by external factors such as nutrition (Smith et al., 2018).

1.4.2.2.4 General considerations on the assessment of physiology to inform welfare

The collection of physiological evidence of compromised welfare (HPA and ANS activity, and immune function) can provide useful information concerning whether sheep are having difficulty coping with challenges, but they are arguably the least feasible under commercial conditions. Measures for on-farm application need to be practical and non-intrusive, not to mention integrative and robust (Webster, 2005b). Even attempting to gather one of the less invasive measures (such as HR and HRV– requiring the gathering, handling, and restraining of all stock at multiple time points), would be difficult, if not impossible, in most production contexts. In addition, the disturbance of animals in such ways (e.g. handling, gathering and restraint) complicates the interpretation of results limiting their usefulness. A second reason for finding physiological measures unsatisfactory for commercial application is that they are costly and often delayed, requiring external analysis (e.g. immunological measures). Finally, there is the potential for physiological data to be misinterpreted or misleading in that changes may actually reflect normal physiological adjustments to ensure homeostasis (Wiepkema and Koolhaas, 1993).

From an animal welfare standpoint, some physiological indices (e.g. cortisol; Mormède et al., 2007) are also lacking in that they do not provide detailed information concerning the affective state of the animal – they may not reflect the intensity nor the valence (positive or negative) of response. An important problem is that the physiological responses are general or 'non-specific', with similar responses occurring under a wide range of situations (Table 1.4). Given these limitations, it is unsurprising that few physiological measures are included in established welfare assessment protocols such as Welfare Quality® and AWIN.

1.4.2.3 Behavioural measures

Behavioural analysis can reflect challenges to an animal's homeostasis and therefore animal welfare, with physical and psychological challenges evoking behavioural responses directed towards removing the challenge and restoring the animal's optimal state (Mench, 1998; Reeder and Kramer, 2005; Webster, 2005a). The initial response when challenged is one of orientation, designed to locate and evaluate the threat (Broom and Johnson, 2019b). This may then be followed by the startle and/or 'fight-or-flight' responses, and these behaviours can be used to indicate that an animal is facing a challenge (Fraser and Broom, 1990). Importantly, behaviour can be used to assess the human-animal relationship (HAR) which is an important component of welfare, especially in extensive systems where human-animal interactions are infrequent (reviewed by; Waiblinger et al., 2006). Behaviour can also be used in the early detection or pre-clinical diagnosis of health issues (Rutherford, 2002; Dawkins, 2004; Gougoulis et al., 2010). Consequently, behavioural responses are not just actions taken when faced with a challenge (i.e. corrective responses) but include an animal's normal actions employed to meet their needs for physical and psychological health (e.g. grazing, social and grooming behaviours), or to prevent injury, illness or negative experiences (i.e. avoidance responses), while abnormal patterns (e.g. abnormal posture and movement, stereotypies, redirected behaviour) can reflect that an animal is having difficulty coping. Therefore, all such behaviours may meaningfully inform welfare. Although a plethora of behavioural indices is available that can be used to evaluate and study animal welfare, it is beyond the scope of this review to discuss all the behavioural measures available in their entirety, instead, some of the key behaviours that are frequently employed to assess the welfare state of animals will be considered, alongside some more novel assessments that could be useful in practical situations.

1.4.2.3.1 Quantitative methods to capture animal behaviour

1.4.2.3.1.1 Ethograms and modified behaviour to inform welfare

Ethograms and time budgets provide an important behavioural means to evaluate stress and welfare in animals. Ethograms describe and capture the full repertoire of major physical activities and movements, identified by observation, and are often used to construct time budgets in animals (Fraser and Broom, 1990; Mench and Mason, 1997; Wemelsfelder, 1997; Webster, 2005a). Time budgets – measures of the duration of particular behaviours – can provide extensive information about the health and welfare of animals (Webster, 2005a). For example, evaluation of the time spent on feeding and grazing can be used as indicators of gastrointestinal abnormalities (Gougoulis et al., 2010) or pain (Fell and Shutt, 1989) in sheep. Furthermore, sick animals are likely to be less active to conserve energy for costly immune responses such as fever (Hart, 1988; Aubert, 1999), and this inactivity may be reflected in animal time budgets. Continuous time budgets on individual animals, although detailed, may be somewhat limited in their appropriateness for practical application given that they are both time and resource consuming, requiring detailed observation of an animal for an extended period of time (Webster, 2005a; Barrell, 2019). However, time budgets can also be collected using an instantaneous or scan sampling approach, where the behaviour of an individual animal or a group of animals is recorded at pre-determined intervals. This approach is more suited for practical application. Overall, such data is valuable and can be used to indicate where there a welfare-relevant changes in behavioural patterns (Table 1.5). For example, during heat stress, sheep were less active, grazing less during the day and seeking shade (Silanikove, 2000), while lambs of dams with subclinical mastitis altered their suckling behaviour and preferentially approached and suckled at healthy mammary glands (Gougoulis et al., 2008).

Table 1.5. Examples of changes in general activity or behaviour of sheep that may inform welfare through measurement of duration or percentage of time spent performing each behaviour as derived from the literature.

Change in behaviour	Cause	References
Feeding		
↓	Inappetence	Rice et al. (2016)
↓	Gastrointestinal parasites	Hutchings et al. (2000)
↓	Pain caused by husbandry procedures	Fell and Shutt (1989)
↓	Isolation	Cockram et al. (1994)
Rumination		
↓	Water deprivation	Gordon (1965)
↓	Isolation	De et al. (2018)
↓	Lameness	Ibrahim et al. (2018)
↓	High ambient temperature	De et al. (2017)
↓	Transport	Cockram et al. (2004)
↓	High level handling regime	Sutherland et al. (2016)
↓	Food (grazing) restriction	Chen et al. (2013)
↑	Pleasure caused by brushing	Tamioso et al. (2018)
Drinking		
↓	Pain caused by husbandry procedures	Edwards et al. (2011)
↑	High ambient temperature	Paranhos da Costa et al. (1992)
Lying		
↓	Social mixing	Sevi et al. (2001b)
↓	Sheep scab (*Psoroptes ovis*)	Berriatua et al. (2001)
↓	Shearing	Hutchinson and McRae (1969)
↓	Food restriction	Yurtman et al. (2002)
↑	Comfort	Hansen (2015)
↑	Lameness	Hodgkinson (2010)
↑	Anaemia caused by *Haemonchus contortus*	Risso et al. (2015)
Standing		
↑	Heat stress	Pent et al. (2019)
Walking		
↓	Pain caused by husbandry procedures	Edwards et al. (2011)
Abnormal standing, walking, and lying		
↑	Pain caused by husbandry procedures	Grant (2004)

The recent advancement of biosensors may overcome the laborious limitations associated with continuous time budget assessments. Numerous wearable technologies that could help manage animal health are increasingly available, including those to measure T_b, detect sweat constituents, detect pathogens or viruses, and record behaviour and movement (reviewed in general by; Neethirajan, 2017; and in sheep by; Fogarty et al., 2018). For example, the recording and discrimination of an animal's daily behavioural activity are made possible with the use of tri-axial accelerometers attached to the animal via collar or halter to the neck or jaw, tag to the ear or mounted to the leg (Table 1.6). While it appears that most of the research on biosensors to record behaviour have been conducted in cattle, they have been used for basic identification and discrimination of locomotive (walking, running) and feeding (grazing, suckling, ruminating) behaviours alongside active and inactive behaviours, and more recently, to identify lameness in sheep (Table 1.6). Validity has not yet been fully evaluated and concerns over the reliability and accuracy of such sensors have been raised with misclassifications of behaviour evident in some studies (e.g. Marais et al., 2014; Fogarty et al., 2020). Furthermore, a period of time may be required for sheep to become accustomed to such biosensors.to ensure that they do not affect the animal and thus the results. However, advancements will likely allow for the more detailed and comprehensive recording and discrimination of an animal's daily activity in the future. In terms of commercial application, such sensors would be invaluable for on-farm welfare assessments in sheep to detect changes in locomotor and grazing activity that may indicate injuries, disease, or predation.

Table 1.6. Summary of studies that have validated biosensors to record locomotive behaviour in sheep as derived from the literature. Type, attachment, and accuracy of sensor/s are also specified.

Behavioural category	Type of sensor/s	Attachment	Reported accuracy*	Reference
General activity				
	Tri-axial accelerometer	Jaw	HIGH	Alvarenga et al. (2016)
	Tri-axial accelerometer	Collar, front-leg, and ear	MEDIUM to HIGH	Barwick et al. (2018b)
	Tri-axial accelerometer	Collar, front-leg, and ear	LOW to HIGH (ear > collar > leg)	Barwick et al. (2020)
	Tri-axial accelerometer	Ear	MEDIUM to HIGH	Fogarty et al. (2020)^
	Tri-axial accelerometer and gyroscope sensor	Ear and collar	HIGH	Walton et al. (2018)
Gait and posture				
	Tri-axial accelerometer	Hind-leg	HIGH	Radeski and Ilieski (2017)
Lame vs. sound locomotion				
	Tri-axial accelerometer	Collar, front-leg, and ear	LOW to MEDIUM (ear > collar > leg)	Barwick et al. (2018a)
	Tri-axial accelerometer and gyroscope sensor	Ear	MEDIUM	Kaler et al. (2020)
Feeding behaviour				
	Tri-axial accelerometer	Head	MEDIUM to HIGH	Mason and Sneddon (2013)
	Tri-axial accelerometer	Jaw	HIGH	Alvarenga et al. (2020)
	Tri-axial accelerometer and gyroscope sensor	Ear and collar	HIGH	Mansbridge et al. (2018)
	Tri-axial accelerometer and gyroscope sensor	Collar	HIGH	Guo et al. (2018)
	Tri-axial accelerometer	Jaw	HIGH	Giovanetti et al. (2017)£
Suckling behaviour				
	Tri-axial accelerometer	Collar	HIGH	Kuźnicka and Gburzyński (2017)
Active vs. inactive behaviour				
	Pitch and roll tilt sensors	Collar	HIGH	Umstätter et al. (2008)
	Omnidirectional accelerometer (Actiwatch)	Collar	LOW to MEDIUM	McLennan et al. (2015)
Rest-activity cycles and sleep pattern				
	Omnidirectional accelerometer (Actiwatch)	Neck	–	Rurak et al. (2008)

* Accuracy: LOW < 50%; MEDIUM = 50-90%; HIGH > 90%; − Information not available.
^ Body posture was also assessed using the accelerometer in this study.
£ Bite frequency was also assessed using the accelerometer in this study.

Advancements in biosensors may facilitate the monitoring of behavioural synchrony – the degree of conforming behaviour observed between individuals within a group at the same time. Synchrony is a measure of social cohesion (Asher and Collins, 2012) and has been suggested to be a useful indicator of positive welfare in ruminants (see; Mattiello et al., 2019). Synchrony in sheep is not yet well studied, but as a gregarious species, sheep display a high level of synchrony in their active (foraging/grazing) and inactive (resting/ruminating) behaviours (e.g. Rook and Penning, 1991; Gautrais et al., 2007). A small number of studies have investigated synchrony in housed sheep (e.g. Bøe et al., 2006; Jørgensen et al., 2009a; Jørgensen et al., 2009b), and there is some limited evidence that smaller housing systems, that restrict lying space, reduce lying synchrony and increase physical displacements in ewes (Bøe et al., 2006). There is also evidence that synchrony may increase in sheep when given more space at pasture (e.g. Hauschildt and Gerken, 2016). However, gregariousness may be a personality trait that differs between individual sheep, one that may play a role in the level of synchrony achieved by the group (Hauschildt and Gerken, 2015). The validity, reliability, and usefulness of the measurement of synchrony in sheep kept in extensive systems have yet to be evaluated, and investigations would have to determine if welfare state plays a role in their synchrony. Biosensors may help determine the level of synchrony between sheep within a flock under extensive systems replacing the need for instantaneous scan sampling of animals.

1.4.2.3.1.2 The performance of specific behaviours to inform welfare

Sheep welfare state may be evident through locomotive behaviours and postures, social behaviours, ear postures, pain-related behaviours and postures, stereotypic behaviour, and feeding, drinking and elimination (Table 1.7). For example, vocalisations have been used in assessments of sheep in various situations, including the presence of predators, separation from conspecifics, in response to pain, habituation to stimuli, and positive human interaction (Table 1.7). Assessments of these behaviours can be obtained by recording: i) the presence and absence of particular behaviour; ii) the frequency of occurrence of each behaviour during an observational period; iii) the latency to perform a particular behaviour; iv) the duration of each occurrence of a particular behaviour; v) the duration between particular behaviours; and vi) the intensity of the behaviour at each occurrence such as the speed of movement (Martin and Bateson, 1986; Fraser and Broom, 1990; Mills and Marchant-Forde, 2010). However, work on behavioural indicators of welfare in sheep are largely from observations taken during controlled experiments, and their validity, reliability and feasibility under less controlled, practical settings needs to be evaluated.

Table 1.7. Examples of the major groups of quantitative behavioural indicators of physical and psychological state that may inform welfare in sheep as derived from the literature. Examples of situations in which the behaviours have been studied are specified. Disease-specific behaviours are reported elsewhere.

Behaviour	Evoking stimuli or conditions												References
	Presence or threat of predator	**Sudden, aversive, or uncontrollable event**	**Isolation**	**Separation from conspecifics**	**Food deprivation**	**Social mixing**	**Restricted housing conditions**	**Barren environment**	**Pain**	**Habituation to stimuli**	**Enrichment**	**Positive human interaction***	
Locomotive behaviours													
Vigilance behaviours	■	■											Baldock and Sibly (1990); Bouissou and Vandenheede (1995); Vandenheede et al. (1998); Beausoleil et al. (2005); Lee et al. (2016)
Immobility	■		■	■									Bouissou and Vandenheede (1995); Vandenheede et al. (1998); (Viérin and Bouissou, 2003); Lee et al. (2016)
Escape attempts	■	■	■	■									Price and Thos (1980); Romeyer and Bouissou (1992); Greiveldinger et al. (2009); (González et al., 2013)
Exploration	■		■	■						■			Beausoleil et al. (2005); (Erhard et al., 2006; Pedernera-Romano et al., 2010; González et al., 2013)
Locomotive activity	■	■	■	■						■			Torres-Hernandez and Hohenboken (1979); Romeyer and Bouissou (1992); Vandenheede et al. (1998); (Carbajal and Orihuela, 2001)
Social behaviours													
Vocalisations^	■	■	■	■	■	■			■	■		■	(Torres-Hernandez and Hohenboken, 1979; Price and Thos, 1980; Romeyer and Bouissou, 1992; Cockram et al., 1993; Le Neindre et al., 1993;

Behaviour	**Evoking stimuli or conditions**												**References**
	Presence or threat of predator	**Sudden, aversive, or uncontrollable event**	**Isolation**	**Separation from conspecifics**	**Food deprivation**	**Social mixing**	**Restricted housing conditions**	**Barren environment**	**Pain**	**Habituation to stimuli**	**Enrichment**	**Positive human interaction***	
Vocalisations *continued*	■	■	■	■	■	■			■	■		■	Cockram et al., 1994; Bouissou and Vandenheede, 1995; Porter et al., 1995; Vandenheede et al., 1998; Ligout et al., 2002; Tallet et al., 2005; Greiveldinger et al., 2009; Stubsjoen et al., 2009; Rault et al., 2011)
Aggressive behaviours[£]						■	■						Marsden and Wood-Gush (1986); Sevi et al. (2001b); Bøe et al. (2006); Van et al. (2007); Miranda-de la Lama et al. (2012)
Affiliative behaviours						■		■					Miranda-de la Lama et al. (2012); Aguayo-Ulloa et al. (2014); Teixeira et al. (2014)
Play behaviours								■			■		Thornton and Waterman-Pearson (1999); Aguayo-Ulloa et al. (2019)
Ear postures[€]													
Changes in ear posture				■					■			■	Reefmann et al. (2009a); Reefmann et al. (2009c); Guesgen et al. (2016b); Tamioso et al. (2018)
Proportion of time spent in ear postures				■					■				Cockram et al. (1993); Reefmann et al. (2009a); Reefmann et al. (2009c); Stubsjoen et al. (2009); Coulon et al. (2015); Tamioso et al. (2017)
Pain-related behaviours[€]													
Active pain avoidance behaviours									■				Molony and Kent (1997); Landa (2003); Grant (2004); Small et al. (2018a)

Behaviour	Evoking stimuli or conditions												References
	Presence or threat of predator	**Sudden, aversive, or uncontrollable event**	**Isolation**	**Separation from conspecifics**	**Food deprivation**	**Social mixing**	**Restricted housing conditions**	**Barren environment**	**Pain**	**Habituation to stimuli**	**Enrichment**	**Positive human interaction***	
Pain postures									■				Lester et al. (1996); Thornton and Waterman-Pearson (1999); Small et al. (2018a); Inglis et al. (2019)
Stereotypic behaviours[€]													
Oral stereotypies					■	■	■				■		Lauber et al. (2012) Cooper et al. (1994) Karaağaç et al. (2005) Vasseur et al. (2006); Miranda-de la Lama et al. (2012); Aguayo-Ulloa et al. (2014); Aguayo-Ulloa et al. (2019)
Other stereotypies							■						Done-Currie et al. (1984)
Other behaviours													
Elimination behaviours	■	■	■	■						■			Romeyer and Bouissou (1992); (Kilgour and Szantar-Coddington, 1997; Carbajal and Orihuela, 2001); Beausoleil et al. (2005); (Erhard et al., 2006)
Tail wagging												■	Grant (2004); Tamioso et al. (2017); Tamioso et al. (2018)
Defence behaviours	■												Torres-Hernandez and Hohenboken (1979); Beausoleil et al. (2005); Early et al. (2020)

[*] Includes brushing, grooming, or stroking.
[^] Includes both high-pitched vocalisations and vocalisations of unspecified pitch.
[£] Includes antagonistic and displacement behaviours
[€] For full list and details of i) ear postures; ii) pain-related behaviours and postures in lambs; and iii) stereotypic behaviours see Table 1.10, Table 1.8 and Table 1.11, respectively

The assessment of behaviour to identify negative affective states, such as fear and pain in sheep, has received particular attention in the literature, with a large number of studies investigating behavioural responses of both lambs and mature sheep to a variety of fear-eliciting situations (Table 1.8). Collectively, the outcome of these studies is that various measures of locomotive behaviour (e.g. activity, immobility, and escape attempts) and postures (e.g. vigilance), together with defence behaviour (e.g. foot stamping, escape behaviours and freezing) and high-pitched vocalisations are accepted as general indicators of negative-valence affective states in sheep. At first glance, such behaviour would indeed appear useful, especially since the intensity of response is reduced once animals become acclimated or habituated (e.g. locomotion; Moberg et al., 1980; and vocalisations; Orgeur et al., 1998; Stubsjoen et al., 2009). However, these behaviours are also evident in other situations, and may not necessarily reflect a negative affective state nor a negative welfare state. For example, immobilisation may reflect docility and the absence of fear, or may indicate that the animal is highly disturbed and distressed (Cockram, 2004). Sex, age, and breed are known to influence behavioural responses of sheep to fear (e.g. Le Neindre et al., 1993; Vandenheede and Bouissou, 1993; Viérin and Bouissou, 2003; Horton and Miller, 2011). Previous experiences also play a role in behavioural responses of sheep to fear and stress (see; Dwyer, 2004). Furthermore, since animals may respond to the same situation or stimuli differently, variation between animals can be an issue. For example, Beausoleil et al. (2005) did not report the inhibition of vocalisations in ewes in the presence of humans or dogs, a finding that has been reported by numerous other studies (see Table 1.8). Finally, although these behaviours are valuable in the evaluation of management practices and for experimental purposes, their use to indicate fear or other negative affective states under extensive systems has not yet been studied and may not be appropriate. They appear to be of limited use except perhaps to indicate that animals have been disturbed in some way, which may indicate a predator or an otherwise aversive event.

Table 1.8. Examples of quantitative behavioural indicators of fear in sheep as derived from the literature. The conditions or stimuli that evoke the behavioural change and the age of study sheep are specified.

Behavioural indicator	**Evoking stimuli/condition(s)**	**Age group**	**References**
Vigilance or orientation behaviour towards stimuli			
↑	Aversive, uncontrollable, or sudden event	Lambs and adults	Vandenheede et al. (1998); Greiveldinger et al. (2009)
↑	Isolation, separation from conspecifics or reduced group size	Lambs and adults	Cockram et al. (1994); Dumont and Boissy (2000)
↑	Presence of a predator (dog and/or human)	Adults	Baldock and Sibly (1990); Bouissou and Vandenheede (1995); Beausoleil et al. (2005); Lee et al. (2016)
↑	Threat of predator (dog) after stimuli removed	Adults	Lee et al. (2016)
Escape attempts (e.g. charges at wall, jumping, rearing against wall)			
↑	Aversive, uncontrollable, or sudden event	Lambs and adults	Romeyer and Bouissou (1992); Greiveldinger et al. (2009)
↑	Presence of a human	Lambs and adults	Romeyer and Bouissou (1992)
↑	Isolation or separation from conspecifics	Lambs	Price and Thos (1980); González et al. (2013); Mora-Medina et al. (2017)
↓	Presence of a human	Lambs	Price and Thos (1980)
Immobility/freezing			
↑	Presence of a human	Lambs and adults	Bouissou and Vandenheede (1995); Vandenheede et al. (1998)
↑	Presence of a predator (dog)	Adults	Lee et al. (2016)
Locomotive activity (e.g. number of squares crossed, number of steps taken, distance travelled, speed of movement)			
↑	Sudden or surprise event	Lambs and adults	Romeyer and Bouissou (1992); Vandenheede et al. (1998)
↑	Exposure to novel object	Lambs and adults	Romeyer and Bouissou (1992)
↑	Isolation or separation from conspecifics	Lambs and adults	Torres-Hernandez and Hohenboken (1979); Kilgour and Szantar-Coddington (1997); Vandenheede et al. (1998); Carbajal and Orihuela (2001); González et al. (2013)
↓	Presence of a human	Lambs and adults	Romeyer and Bouissou (1992)
↓	Presence of a predator (dog)	Adults	Torres-Hernandez and Hohenboken (1979)
Vocalisations*			
↑	Aversive and uncontrollable event	Lambs	Greiveldinger et al. (2009)
↑	Separation of ewe and lamb	Adults	Cockram et al. (1993); Porter et al. (1995); Orgeur et al. (1998)

Behavioural indicator	Evoking stimuli/condition(s)	Age group	References
↑	Sudden event	Lambs and adults	Romeyer and Bouissou (1992); Greiveldinger et al. (2009)
↑	Isolation or separation from conspecifics	Lambs and adults	Moberg et al. (1980); Baldock and Sibly (1990); Cockram et al. (1994); Poindron et al. (1997); Vandenheede et al. (1998); Deiss et al. (2009); Rault et al. (2011)
↑	Presence of a goat	Adults	Beausoleil et al. (2005)
↑	Presence of a human	Lambs	Viérin and Bouissou (2003)
↓	Presence of a human	Lambs and adults	Price and Thos (1980); Romeyer and Bouissou (1992); Le Neindre et al. (1993)
↓	Presence of a predator (dog)	Adults	Torres-Hernandez and Hohenboken (1979)
Foot stamping			
↑	Presence of a predator (dog)	Adults	Torres-Hernandez and Hohenboken (1979); Beausoleil et al. (2005); Early et al. (2020)
Pawing			
↑	Isolation	Lambs	Cockram et al. (1994)

* Includes both high-pitched vocalisations and vocalisations of unspecified pitch.

For the identification of pain in sheep, numerous pain-related behaviours are evident in lambs following painful routine husbandry procedures (Table 1.9) and a simple numerical pain scale (0-3) has been developed and validated to assess pain in lambs (Lomax et al., 2008; Lomax et al., 2010; Lomax et al., 2013). Pain disrupts lying and feeding behaviours of sheep (e.g. Hemsworth et al., 2009; Edwards et al., 2011) and may also lead to the expression of other behaviours such as teeth grinding, trembling and lip curling (Molony et al., 1997; Dobromylskyj et al., 2000), although such behaviours are not common in lambs following painful husbandry procedures (Grant, 2004), or they are difficult to distinguish, possibly masked by other movements (e.g. Lester et al., 1996; Molony et al., 2002). Furthermore, inconsistencies between studies that use these behaviours to assess pain are evident, and there are difficulties in distinguishing different types of pain (e.g. ischemic and inflammatory, visceral, and somatic or superficial, and neuropathic pain). For example, the adoption of abnormal standing postures following castration found by Molony et al. (1993) was not evident in the research by Grant (2004). Another challenge is that some behaviours do not necessarily indicate pain. For example, increases in tail wagging are evident in lambs following some husbandry procedures, but are also observed in lambs during suckling (Grant, 2004) and when they are brushed (Tamioso et al., 2017; Tamioso et al., 2018). Such behaviours may be useful indicators of pain in lambs under commercial conditions, but caution should be used when interpreting changes in terms of welfare.

Importantly, it appears that behavioural indicators for pain in mature sheep are lacking. Indeed, outside of clinical observations of sheep responses in studies designed to assess post-operative pain and pain amelioration (e.g. Otto et al., 2000; Kania et al., 2006; Faure et al., 2017), it appears that only the responses of mature sheep to presumably painful diseases such as lameness (e.g. Barwick et al., 2018a; Doughty et al., 2018; Kaler et al., 2020) and mastitis (e.g. Gougoulis et al., 2008; McLennan et al., 2016) have been studied in any depth under practical settings. From these studies comes perhaps the single most widely adopted behavioural indicator in commercial conditions: the scoring of locomotion to identify lameness (see; Kaler and Green, 2008). In most cases, lameness in sheep is due to bacterial infection, lesions and foot-related diseases (Raadsma and Egerton, 2013; Gelasakis et al., 2019) and is a painful condition (Ley et al., 1989). This pain can cause deviations in normal gait, which observers can reliably identify and quantify using the seven-point scoring system (Kaler and Green, 2008; Kaler et al., 2009). As such, lameness scoring is an extremely valuable tool available to producers to improve welfare on-farm through prompt identification and treatment of lame animals and to prevent additional pain.

A more novel approach to the assessment of pain in sheep is through facial expressions. Recent efforts have produced sheep and lamb grimace, facial action unit, and facial expression scoring systems to assess pain in sheep. These facial expression scoring systems have been successfully applied to distinguish pain caused by footrot and mastitis (McLennan et al., 2016), unilateral osteotomy of the right hind leg (Häger et al., 2017), and tail docking (Guesgen et al., 2016a). There is potential for automation of facial expression scoring using computer vision techniques and machine learning (Lu et al., 2017; McLennan and Mahmoud, 2019). Furthermore, it is perhaps reasonable to propose that if facial expression scoring systems can be developed for pain, that other subjective experiences in sheep may also be quantified in this manner. For example, Defensor et al. (2012) studied the facial expressions of mice beyond pain, describing the facial expressions of mice under fear and aggressive contexts (the presence of social, non-social and predator stimuli). Likewise, Bremhorst et al. (2019) evaluated the facial expressions of dogs in response to positive anticipation of food and also frustration, finding that differences in facial expression may help distinguish the valence of emotion. While advances have been made, this concept is still in its preliminary stages and does not yet appear to have been applied to assess pain in sheep outside those studies by McLennan et al. (2016), Häger et al. (2017), and Guesgen et al. (2016a). These scoring systems must be thoroughly investigated to ensure validity, reliability and feasibility before they can be used under commercial conditions for remote capture with the help of surveillance technology (for further information on the use and validity of facial expression scales in mammals see; McLennan et al., 2019).

Table 1.9. Pain-related behaviours and postures in lambs in response to painful routine husbandry procedures as derived from the literature.

Behaviour	Husbandry procedure*	References
Active pain avoidance behaviours		
Foot stamping/kicking	ET, C, TD, CTD	Kent et al. (1998); Kent et al. (2000); Molony et al. (2002); Mellema et al. (2006); McCracken et al. (2010); Paull et al. (2012); Guesgen et al. (2014); Futro et al. (2015); Karakuş and Karakuş (2017)
Tail wagging	C, TD, CTD	Kent et al. (2000); Molony et al. (2002); Landa (2003); Futro et al. (2015)
Easing quarters	C, TD	Kent et al. (1998); McCracken et al. (2010); Paull et al. (2012); Futro et al. (2015)
Head turning/shaking	ET, C, TD, CTD	Kent et al. (1998); Kent et al. (2000); Edwards et al. (2001); Molony et al. (2002); Landa (2003); Futro et al. (2015); Karakuş and Karakuş (2017)
Head butting	C	Guesgen et al. (2014)
Restlessness	C, CTD	Molony et al. (1993); Lester et al. (1996); Kent et al. (1998); Mellema et al. (2006); Paull et al. (2012)
Lip curling	C, CTD	Molony et al. (2002)
Licking and biting wound site	C, TD, MTD	McCracken et al. (2010); Colditz et al. (2012); Paull et al. (2012); Small et al. (2018b)
General increase in active behaviours	C, TD, CTD, MTD, MCDT	Molony and Kent (1997); Landa (2003); Grant (2004); Small et al. (2018a)
Pain postures		
Abnormal ventral lying	C, CTD	Molony et al. (1993); Molony and Kent (1997); Molony et al. (2002); Colditz et al. (2012); Paull et al. (2012); Futro et al. (2015)
Abnormal lateral lying	C, CTD	Molony et al. (1993); Molony et al. (2002); Futro et al. (2015)
General increase in abnormal lying^	C, CTD	Kent et al. (1998); Kent et al. (2000)
Abnormal standing (including hunched standing)	C, CTD, M, MTD, MCDT	Fell and Shutt (1989); Molony et al. (1993); Chapman et al. (1994); Kent et al. (1998); Kent et al. (2000); Molony et al. (2002); Grant (2004); Paull et al. (2007); Paull et al. (2008); Hemsworth et al. (2009); Colditz et al. (2012); Hemsworth et al. (2012); Paull et al. (2012); Futro et al. (2015); Small et al. (2018b)
Abnormal walking	MTD	Paull et al. (2007)
General increase in abnormal postures^	C, TD, CTD, MTD, MCTD	Lester et al. (1996); Thornton and Waterman-Pearson (1999); Small et al. (2018a); Inglis et al. (2019)

* Husbandry procedure; ET = ear tagging; C = castration; TD = tail docking; CTD = combined castration and tail docking; M = mulesing; MTD = combined mulesing and tail docking; and MCTD = combined mulesing, castration, and tail docking.

^ Breakdown of abnormal postures not specified

Outside of pain and fear, the study of psychological states in sheep using behaviour has not received much attention, presumably because of the general challenges associated with assessing affective state in animals. However, recently, ear postures have emerged as a novel means to assess psychological state in sheep, and studies have investigated ear posture to evaluate negative as well as positive affective states in sheep (Table 1.10). Negative stimuli such as pain and separation from conspecifics elicit more frequent changes in ear posture, whereas sheep subject to positive stimuli such as brushing, grooming, and feeding have fewer changes (Table 1.10). The predominant ear posture exhibited by sheep is also thought to provide meaningful information in the assessment of affective state and welfare (Table 1.10); however, more validation work is required since responses may not be consistent (e.g. Raoult and Gygax, 2018) and specific postures may be associated with either positive or negative stimuli. For example, the raised ear posture was the predominant posture in lambs anticipating brushing (Tamioso et al., 2017) but also in sheep exposed to an unfamiliar and unpleasant situation (Boissy et al., 2011). It may also be difficult to reliably capture ear posture changes without the use of video recording equipment if shifts occur rapidly; even with such technology, some authors have found ear postures too difficult to observe and assess accurately (e.g. Anderson et al., 2015).To accurately recognise ear postures, assessors need a clear view of the animal’s head and both ears, which may be difficult to achieve under commercial conditions.

Table 1.10. Summary of studies that investigate ear posture in sheep to evaluate affective state. Evoking stimuli and the valance of presumed affective state elicited are specified.

Ear related behaviour	Evoking stimuli	Valance of presumed affective state	References
Ear posture changes			
↑	Separation from conspecifics	Negative	Reefmann et al. (2009a); Reefmann et al. (2009c)
↑	Pain caused by husbandry procedures	Negative	Guesgen et al. (2016b)
↓	Brushing or grooming	Positive	Reefmann et al. (2009c); Tamioso et al. (2018)
↓	Feeding	Positive	Reefmann et al. (2009a)
Proportion of axial or hanging (passive) ear postures			
↑	Stroking	Positive	Reefmann et al. (2009b); Coulon et al. (2015)
↑	Brushing or grooming	Positive	Reefmann et al. (2009c); Tamioso et al. (2017)
↑	Feeding	Positive	Reefmann et al. (2009a)
↑	Neutral event*	Neutral	Boissy et al. (2011)
Proportion of forward, back or raised ear postures			
↑	Separation from conspecifics	Negative	Reefmann et al. (2009a)
↑	Separation of ewe and lamb	Negative	Cockram et al. (1993)
↑	Smaller food reward than expected	Negative	Boissy et al. (2011)
↑	Novel and sudden event	Negative	Boissy et al. (2011)
↑	Aversive event	Negative	Greiveldinger et al. (2009)
↑	Pain caused by husbandry procedures	Negative	Guesgen et al. (2016b)
↑	Pain caused by noxious ischaemic stimulus	Negative	Stubsjoen et al. (2009)
↑	Anticipation of brushing event	Positive^	Tamioso et al. (2017)

* Animals already had training - repeated exposure to stimuli, or undisturbed in this situation.
^ Raised ears only. Lambs expressed more raised ears pre-brushing which the authors suggested related to anticipation of brushing.

Stereotypic behaviour – repetitive and unvarying behavioural patterns that do not serve an obvious goal or function (Fraser and Broom, 1990; Mason, 1991b) – is commonly believed to indicate poor welfare in animals when stereotypic behaviour accounts for more than 10% of an animals daily activity (see; Broom, 1983). For example, under intensive or confined conditions, sheep demonstrate oral and locomotive stereotypies including wool biting, pacing and the chewing/biting/licking of fixtures. However, these behaviours appear to be exclusively studied in sheep under intensive or confined conditions (Table 1.11), or have been reported absent once animals that have been confined within indoor, restrictive enclosures, are ‘turned out’ (Fraser and Broom, 1990; EFSA, 2012), which limits to usefulness of this stereotypy as an indicator of poor welfare in extensively managed sheep. It is thought that animals develop stereotypic behaviour as a means of coping with inadequacies in their environment that restrict their ability to perform highly-motivated behaviours, such as eating (Edwards, 2010). In this way, stereotypies indicate both sub-optimal environment and frustration, thus poor welfare, but importantly may also reflect that these animals are attempting to cope with their environment, challenging the simplistic interpretation of stereotypies as an indicator of welfare issues (see; Dawkins, 2003; Mason and Latham, 2004). Also, the degree of stereotypy an animal expresses does not necessarily correspond to the degree to which welfare is compromised, and stereotypies may even persist as habit-like behaviour expressed in situations where welfare is not compromised (Mason, 1991a). Interpretation and lack of evidence in sheep under extensive production systems results in limited usefulness of stereotypic behaviour as an indicator of poor welfare in extensively managed sheep.

Table 1.11. Stereotypic behaviours in sheep as derived from the literature. Experimental conditions and specific stereotypic behaviour found are specified.

Study	**Experimental conditions** – **Housing (size)**	**Stereotypic behaviour** – **Oral – Chewing/licking fixtures**	**Oral – Wool biting**	**Locomotive – Pacing /weaving**	**Other – Body rubbing**	**Stereotypic behaviours***
Cooper and Jackson (1996)	Inside – Individual pens (1.5 m^2) and pen of 12 animals (16 m^2)	■	■			
Vasseur et al. (2006)	Inside – Pens of 10 animals (12.5 m^2)		■			
Lauber et al. (2012)	Inside – Individual pens (1.2 m^2 or 1.5 m^2)	■		■		
Yurtman et al. (2002)	Inside – Individual pens (1.2 m^2)	■				
Galvani et al. (2010)	Inside – Individual stalls (1.5 m^2)					■
Marsden and Wood-Gush (1986)	Inside – Individual pens (1.8 m^2)	■	■			
Miranda-de la Lama et al. (2012)	Inside – Pens of 12 animals (9 m^2)	■			■	
Aguayo-Ulloa et al. (2014)	Inside – Pens of 10 animals (9.6 m^2)	■				
Aguayo-Ulloa et al. (2019)	Inside – Individual pens (1.8 m^2)	■				
Savas et al. (2001)	Inside – Pens of 10 animals £	■	■			
Teixeira et al. (2014)	Pens of 6 animals (5.6 m^2) ^	■				
Done-Currie et al. (1984)	Inside – Pens of 3-9 animals (10.4 m^2) or individual pens (1.3 m^2)	■	■	■		
Cooper et al. (1995)	Inside – Individual pens £					■
Cooper et al. (1994)	Inside – Individual pens (2 m^2)					■
Karaağaç et al. (2005)	Outside – Paddock of 10 animals (12 m^2)	■	■			

* Specific stereotype information not available.
^ Inside or outside feedlot pens not specified.
£ Size not available.

Social behaviours such as play and aggression may provide meaningful information relevant to the welfare of sheep. The level of play is a valuable welfare measure with the presence of play reflecting good welfare while the absence of play reflects poor welfare (Boissy et al., 2007; Held and Špinka, 2011). For example, lambs displayed play behaviour before castration but not following the procedure (Thornton and Waterman-Pearson, 1999), while lambs that were prevented from suckling displayed less social behaviours including playing than those allowed to suckle (Napolitano et al., 2003). Importantly, a rebound effect in observed in ruminants, where play is reported to re-commence once conditions improve (see; Mattiello et al., 2019). As such, play could reflect both an improvement in conditions and positive welfare and provides an indication that previous conditions were inadequate or poor. Furthermore, lambs may play or interact positively with environmental enrichment objects (e.g. Aguayo-Ulloa et al., 2019). Play is relatively easy to recognise, although sex and age differences in expression of play are evident in sheep (Sachs and Harris, 1978; Fraser and Broom, 1990). An assessment of play in lambs is valuable, being one of only a few indicators to assess positive welfare (Boissy et al., 2007), but play is uncommon in yearling or older sheep (Fisher and Matthews, 2001). It is also important to note that the absence of play in lambs during observation does not necessarily indicate poor welfare (EFSA 2012).

Aggression or antagonistic interactions between conspecifics may reflect poor welfare, suggesting competition for a restricted resource (e.g. shelter, space, or food) and that some needs of the animals are not being met. Furthermore, aggressive interactions may cause injury, which is a welfare concern in its own right. Aggression is of limited use in sheep as, outside of some male-male interactions, where aggression may reflect dominance relationships (Fisher and Matthews, 2001), sheep at pasture do not appear to be overly aggressive. Indeed, aggressive interactions or fighting are seldom evident in grazing ewes (e.g. Arnold and Maller, 1974; Lynch et al., 1989; Bojkovski et al., 2014), and antagonistic and displacement behaviours are generally only reported in housed sheep when lying space is severely restricted (e.g. Marsden and Wood-Gush, 1986; Bøe et al., 2006) or for a short period of time in housed animals when unfamiliar animals are first mixed (e.g. Sevi et al., 2001b; Miranda-de la Lama et al., 2012; Aguayo-Ulloa et al., 2014).

Other than play, positive social behaviours – affiliative behaviour – are not commonly expressed in sheep, but when present, may be promising indication of long-term positive affective state in livestock (Boissy et al., 2007). Affiliative behaviour such as grooming or mutual licking, nibbling, and sniffing, are observed during courtship and mating between ram and ewe, and between dam and lamb(s) (Fisher and Matthews, 2001). Some studies have investigated affiliative behaviour in lambs under confined conditions (e.g. Miranda-de la Lama et al., 2012; Aguayo-Ulloa et al., 2014; Teixeira et al., 2014; Liebenberg, 2017), but the interpretation of affiliative behaviours in welfare terms is still not straightforward. For example, increases in affiliative behaviours have been reported in lambs following social mixing (Miranda-de la Lama et al., 2012) and in lambs kept in barren environments (Aguayo-Ulloa et al., 2014; Teixeira et al., 2014), both of which are arguably negative in nature. Consequently, increases in affiliative behaviours may indicate positive experiences, or an animal's attempt to escape negative experiences. For this reason, their infrequent expression, and since the use of affiliative behaviours have yet to be validated except for lambs kept in confined conditions, makes the usefulness of affiliative behaviours doubtful and their adoption for practical use in commercial settings unlikely.

The last category of behaviours for welfare assessment are those related to disease. It is not uncommon for behaviour to be a clinical sign of a pathological condition, and altered behaviour is often the first indication of illness (Fraser and Broom, 1990). In fact, disease in sheep is often detected because of systemic behavioural signs such as depression, social withdrawal, and reduced appetite, or because animals present with characteristic behavioural signs such as ataxia, stargazing posture, or circling (Table 1.12). Overall, behaviour is a valuable diagnostic tool during veterinary examination and could be formally applied in commercial situations to inform and improve welfare. The discussion of all of the behaviours related to the numerous important diseases in sheep would make this review impossibly long; instead, the behavioural signs of some common sheep health issues are presented in Table 1.12, with a discussion of the usefulness of recording general behavioural signs of disease to inform welfare under commercial conditions.

The recording of those general behavioural signs of disease: i) depression; ii) social withdrawal, and iii) reduced appetite, is perhaps the most feasible to collect under commercial conditions given advancements in biosensors and remote capture systems. Depression is a clinical term referring to the reduced general activity, responsiveness of an animal to external stimuli and reduced awareness (Fraser and Broom, 1990), and thus observed reductions in general activity (grazing/walking) may indicate disease and inform welfare. Likewise, social withdrawal, or the isolation of an individual from its group, is often an indication of illness (Constable et al., 2017), and the discovery of a withdrawn sheep is of concern and should be monitored since they are a highly gregarious species (Fisher and Matthews, 2001). Reduced appetite is also an important component of many health issues in sheep (Table 1.12) and knowledge of feeding behaviour may allow the identification of those with reduced or lost appetites (inanition). At first glance, these behaviours appear to be useful indicators of health and thus welfare concerns in sheep; however, the performance of many of these behaviours are common across many diseases (Table 1.12). Thus, care should be given when weighing the importance of and interpreting these behavioural signs in disease and welfare terms. Furthermore, these are arguable impractical to collect under commercial conditions, particularly in extensive systems without the use of biosensors.

Importantly, we cannot currently afford to monitor and record disease-related behavioural data commercially, it would be too time and resource consuming. Yet, perhaps biosensors such as radio-frequency identification (RFID), accelerometers, proximity loggers (PL) and global positioning system (GPS) could be adapted for this purpose. For example, the application of GPS, PL and RFID could be useful to identify social withdrawal. The use of GPS to monitor livestock location is self-explanatory and PL and RFID have already been successfully applied to record ewe-lamb (e.g. Broster et al., 2010) and ewe-ewe interactions (e.g. Freire et al., 2012; Doyle et al., 2016), to measure resource use in sheep kept at paddock (Broster and Doyle, 2013), and to identify inappetent sheep at a pre-embarkment feedlot (Barnes et al., 2018). Thus, it is reasonable to suggest that such monitoring could be used to give advanced warning when declining or total cessation of social interaction and/or feeding is recorded. This is, of course, a novel concept and would need to be thoroughly investigated to ensure validity, reliability, and feasibility.

The recording of these general behaviours in commercial settings may not necessarily aid in the immediate diagnosis of disease but would perhaps provide a red flag to producers signifying that these animals might be in ill-health and warrant a closer inspection, improving action for animal welfare. This is an obvious area in which future work should focus, with issues limiting feasibility and validity needing to be addressed.

Table 1.12. Summary of the behavioural signs of common or important health issues in sheep. Diseases that are routinely vaccinated against in Australia such as tetanus and pulpy kidney are not presented here. Ovine Johne's disease is also not presented as there are no known behavioural signs of infection.

Health issue	Behavioural signs		References
	Clinical signs	Other possible signs	
Mastitis	Depression; and reduced appetite	Altered or stiff gait (may appear lame); and social withdrawal	Fraser and Broom (1990); Menzies and Ramanoon (2001); Hindson and Winter (2002); Watkins and Jones (2007); Zadoks and Duncan (2014)
Pregnancy toxaemia (ketosis)	Reduced appetite; depression; social withdrawal; bleating; teeth grinding; muscle tremors of the head and neck; abnormal head carriage; reluctant to move; and recumbency with inability to rise	Head pressing; stargazing posture; circling; and persistent drinking	Chesney (1956); Schulz and Riese (1983); Marteniuk and Herdt (1988); Andrews (1997); Rook (2000); Navarre and Pugh (2002); Bulgin (2007); Sargison (2007); Lorenz et al. (2011); Scott (2015)
Hypocalcaemia (lambing sickness)	Social withdrawal; temporary stiff gait; muscle tremors; ataxia*; depression; and recumbency with head turned toward flank and inability to rise	Decreased defecation and urination	Scott (1995); Cockcroft and Whiteley (1999); Bulgin (2007); Skyes (2007); Lorenz et al. (2011); Scott (2015)
Hypomagnesaemia (grass tetany)	Depression; stiff gait; reduced appetite; teeth grinding; ear flapping; muscle tremors; and collapse and convulse with legs rigidly extended and head thrown back or stretched out		Chesney (1956); Underwood and Suttle (1999); Foster et al. (2007); Skyes (2007)
Rumen lactic acidosis	Social withdrawal; depression; obtund^; reduced appetite; teeth grinding; and muscle twitching	Ataxia; altered gait; head pressing; and recumbency	Braun et al. (1992); Navarre and Pugh (2002); Bulgin (2007); Snyder and Credille (2017)
Ryegrass toxicity	Staggering gait; ataxia; trembling or convulsions; and collapse		Chesney (1956); Berry and Wise (1975); Trotman (1978); Machen et al. (2002); di Menna et al. (2012)
Copper deficiency (swayback in lambs) and toxicity	Deficiency – uncoordinated and staggering gait; swaying of hindquarters; and ill-thrift Toxicity – depression; social withdrawal; reduced appetite; reluctant to move; and obtund	Deficiency – fine head tremor	Underwood and Suttle (1999); Belknap and Pugh (2002); Navarre and Pugh (2002); Scott (2007b); Scott (2015)

Health issue	Behavioural signs		References
	Clinical signs	Other possible signs	
Cobalt deficiency	Reduced appetite; and ill-thrift		Minson (1990); Ulvund and Pestalozzi (1996); Underwood and Suttle (1999); Ellison (2002); Scott (2015)
Selenium deficiency (white muscle disease or nutritional muscular dystrophy) and toxicity	Deficiency – stiff gait; trembling; reluctant to move; hunched standing; and ill-thrift Toxicity – depression; ill-thrift; stiff gait or lameness; and abnormal appetite		Chesney (1956); Underwood and Suttle (1999); Reilly et al. (2002); (Scott, 2015)
Listeriosis (circling disease)	Depression; ear droop; and inability to eat	Circling; leaning against objects; recumbency; and head tilt	Chesney (1956); Hindson and Winter (2002); Machen et al. (2002); Scott (2007a); Scott (2015)
Scrapie (transmissible spongiform encephalopathy) [¥]	Ataxia of the hindlimbs; head tremor; paralysis; rubbing and biting; and recumbency with inability to rise	Low head carriage; teeth grinding; and circling	Machen et al. (2002); Healy et al. (2003); Jeffrey and Gonzalez (2007); Konold and Phelan (2014)
Polioencephalomalacia (PEM or star gazing disease)	Depression; incoordination; head pressing; social withdrawal; and abnormal or stargazing posture	Convulsions while lying	Machen et al. (2002); Scott (2007b); Scott (2015)
Salmonellosis	Depressed; and reluctant to move		Navarre and Pugh (2002); Perkins et al. (2009); Hoelzer et al. (2011); Navarre et al. (2012)
Coccidiosis	Reduced appetite; and dullness	Ataxia	Reilly et al. (2002); Wright and Coop (2007)
Parasitic gastroenteritis (infection with gastrointestinal helminthosis – excluding *Haemonchus contortus*)	Lethargy; and ill-thrift	Reduced appetite; and abnormal posture (tucked-up belly) [£]	Jackson and Coop (2007); Constable et al. (2017)
Haemochosis (infection with *H. contortus*)	Lethargy; and ill-thrift		Jackson and Coop (2007); Scott (2015)
Fasciolosis (infection with *Fasciola hepatica*)	Depression; and ill-thrift	Reduced appetite	Navarre and Pugh (2002); Mitchell (2007); Miller et al. (2012)
Sheep scab (*Psoroptes ovis*) [€]	Head tossing; and rubbing, scratching, and biting of infected area		Corke and Broom (1999); Berriatua et al. (2001); Hindson and Winter (2002); Bates (2007b)
Lice	Rubbing, scratching, and biting of fleece	Lameness	Anderson et al. (2002); Hindson and Winter (2002); Bates (2007a)

Health issue	Behavioural signs		References
	Clinical signs	Other possible signs	
Flystrike (cutaneous myiasis)	Foot stamping; tail wagging; rubbing; and abnormal posture		Hindson and Winter (2002); Bates (2007a); Constable et al. (2017); Chapter 3 - Grant et al. (2019)
Pneumonia	Depression; social withdrawal; reduced appetite; and cough		Belknap (2002); Ayling and Nicholas (2007); Wilkins and Woolums (2009); Nejiban and Al-Amery (2018)
Footrot	Lameness; grazing on knees; recumbency	Reduced appetite	Reilly et al. (2002); Egerton (2007); Scott (2015)
Arthritis	Lameness of one or more limbs or abnormal gait; and reluctant to move	Reduced appetite; and recumbency	Reilly et al. (2002); Watkins (2007); Scott (2015)

[*] Incoordination of the movements of the body or limbs.
[^] Less than full alertness, similar to lethargy where animal has reduced interest in environment and displays slowed responses to stimuli.
[£] This adoption of this posture is specific to infection with helminth parasite; *Nematodirus battus*.
[€] Sheep scab has been eradicated in Australia.

1.4.2.3.2 Qualitative methods to capture animal behaviour - Qualitative behavioural assessment (QBA)

Traditionally, behaviour has been collected through various quantitative means (as reviewed above); however, qualitative approaches can also be taken. Animal movement and actions have distinctive qualities, where the same or similar behaviours can be done in different manners. Observations of '*how*' animals behave – their behavioural expression, body language or demeanour, can therefore be useful (Fagan et al., 1997). For example, curiosity and fear in sheep are characterised by the same or very similar behavioural actions: alertness and activity (i.e. walking with its head up and ears oriented towards stimuli), which are recognisable in that sheep exposed to a predator threat are ridged and tense, whereas sheep exploring their environment while also active, vigilant and alert, are not (Wemelsfelder and Farish, 2004). In this way, qualitative observations are useful, can help to guide the interpretation of quantitative behaviour and provide information relevant or meaningful to welfare.

Qualitative Behavioural Assessment (QBA) is a 'whole animal' approach to capture the behavioural expression of animals through the integration and summary of details of behavioural events, posture, and movement (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001; Wemelsfelder and Lawrence, 2001). Essentially, it is an approach to assessing '*how*' an animal is behaving rather than '*what*' it is doing, and in this way, captures how an animal interacts with its environment (Wemelsfelder et al., 2001). In brief, the approach involves observation of animals and scoring '*how'* they behave, using a range of descriptive terms such as 'relaxed', 'calm', 'nervous' and 'agitated' to score their behavioural expression along visual analogue scales (VAS) (for a detailed explanation of the methodology see; Wemelsfelder et al., 2000; Wemelsfelder and Lawrence, 2001). This is fundamentally similar to what a good stock person does when they survey their stock, but it formalises observations and captures the body language in numerical terms that can then be analysed statistically (Fleming et al., 2016). By doing this, QBA can provide insight into welfare state of animals (Wemelsfelder, 2007), and may capture subtle differences in the behavioural patterns that may otherwise be missed when behaviour is scored using the more traditional quantitative methods (Fleming et al., 2016). Importantly, since body language is thought to provide an insight into the physical and psychological state of an animal, QBA represents a valuable tool to assess affective state (Boissy et al., 2007; Rutherford et al., 2012; Murphy et al., 2014).

As an assessment tool, QBA is novel. Since its conception, a substantial amount of research has been undertaken to evaluate the methodology for use in welfare assessments (reviewed for application in livestock by; Fleming et al., 2016; and in zoo animals by; Rose and Riley, 2019). Indeed, numerous studies, across several species have investigated the application of the methodology to assess animal welfare (Table 1.13). The majority of these seek to validate the methodology, investigating the ability of observers to distinguish animals in states of compromised welfare from healthy individuals or to assess the welfare of animals under different conditions using either their own set of descriptive terms (free-choice profiling; FCP) or with a pre-determined list of terms (fixed list; FL), from video footage or from live. As with all welfare measures, this is hindered by the lack of a 'gold standard' welfare criterion against which to test validity. Nevertheless, QBA has been validated against numerous measures relevant to welfare across various species, including health status or clinical symptoms (e.g. skin lesions; Camerlink et al., 2016; and mastitis; de Boyer des Roches et al., 2018), behavioural measures (e.g. pain behaviours in response to castration; Vindevoghel et al., 2019) and physiological measures (e.g. heart rate variability, body temperature and white blood cell counts; Wickham et al., 2015). Some studies have also demonstrated the ability of QBA to assess both positive and negative affective states (e.g. Rutherford et al., 2012; Hintze et al., 2017; Serrapica et al., 2017), and several have specifically investigated the inter- and/or intra-observer reliability of the methodology (e.g. Bokkers et al., 2012; Phythian et al., 2016; Czycholl et al., 2017; Diaz-Lundahl et al., 2019). There is evidence that QBA is reliable, and it even appears that assessments made by observers with little experience in the subject species, and those with different backgrounds, can provide valuable information relevant to welfare using this methodology (e.g. Napolitano et al., 2012; Wemelsfelder et al., 2012; Duijvestein et al., 2014). Still, QBA should be used together with other measures (Wemelsfelder and Mullan, 2014), and the interpretation of these assessments require expert opinion and judgment (Fleming et al., 2016).

Table 1.13. Studies that investigate or formally apply qualitative behavioural assessment (QBA) to inform animal welfare.

Species	Reference
Production animals	
Sheep	Cockram et al. (2012); Wickham et al. (2012); Phythian et al. (2013a); Stockman et al. (2013b); Fleming et al. (2015); Wickham et al. (2015); Phythian et al. (2016); Muri and Stubsjøen (2017); Serrapica et al. (2017); Collins et al. (2018); Chapter 6 - Grant et al. (2018); Diaz-Lundahl et al. (2019); Chapter 3 - Grant et al. (2019); Chapter 4 - Grant et al. (2020a); Chapter 5 - Grant et al. (2020b)
Pigs	Wemelsfelder et al. (2000); Wemelsfelder et al. (2001); Temple et al. (2011); Rutherford et al. (2012); Wemelsfelder et al. (2012); Lau (2013); Otten (2013); Duijvestein et al. (2014); Morgan et al. (2014); Munsterhjelm et al. (2015); Camerlink et al. (2016); Clarke et al. (2017); Czycholl et al. (2017); Clarke et al. (2018); Schmitt et al. (2019)
Cattle	Rousing and Wemelsfelder (2006); Knierim and Winckler (2009); Wemelsfelder et al. (2009); Brscic et al. (2010); Stockman et al. (2011); Bokkers et al. (2012); Stockman et al. (2012); Stockman et al. (2013a); de Boyer des Roches et al. (2018); Vindevoghel et al. (2019); Rizzuto et al. (2020)
Goats	Muri et al. (2013); Battini et al. (2016); Grosso et al. (2016); Battini et al. (2018); Miller et al. (2018); Napolitano et al. (2018)
Chickens	Gocsik et al. (2016); Muri et al. (2019)
Horses	Napolitano et al. (2008); Minero et al. (2009); Fleming et al. (2013); Gronqvist et al. (2017); Hintze et al. (2017); Minero et al. (2018)
Donkeys	Dai et al. (2016); Minero et al. (2016)
Buffalo	Napolitano et al. (2012); Napolitano et al. (2015)
Companion and zoo animals	
Dogs	Walker et al. (2010); Walker et al. (2016); Arena et al. (2017); Arena et al. (2019)
Giraffes	Patel et al. (2019)
Elephants	Yon et al. (2019)

Not all studies agree about the validity of QBA as a welfare measures; some studies report poor associations between QBA and other measures of animal welfare (e.g. Andreasen et al., 2013), or report poor reliability (e.g. Bokkers et al., 2012). However, as suggested by Battini et al. (2018), QBA may reflect a different aspect of welfare not necessarily directly related to other measures collected, which may explain poor associations. It should also be noted that, as with other behavioural measures, there is the potential for the behavioural expression of animals to be misunderstood or misinterpreted. Indeed, even if observers agree and reach a consensus in their assessment of an animal's behavioural expression, they can still be collectively wrong in their assessments (Wemelsfelder et al., 2000). For example, Gronqvist et al. (2017) found that observers with little to no previous experience with horses misinterpreted the expressive behaviour of an isolated horse, perceiving the anxious behaviour of the animal as '*curious*', '*playful*', '*happy*' and '*at ease*'. As raised by Fleming et al. (2015) in their study examining the impact of variations in visual and verbal information on observer assessments of sheep using QBA, attention must be given to the training of observers, particularly under less controlled situations such as on-farm. However, studies addressing the impact of intense or continuous observer training on the reliability of QBA assessments, particularly when inexperienced observers are used, appear to be lacking. Lastly, there is some opposition to QBA since it relies on the human ability to perceive and integrate details of behaviour using descriptive terms (e.g. happy, excited, frustrated, and anxious) that have expressive and thus arguably anthropomorphic connotations. However, even though the descriptive terms may have anthropomorphic connotations to the observers using them, these connotations bear no weight in the analysis, and the behavioural patterns they are used to describe are in fact observable, thus valid (Wemelsfelder, 1997; Wemelsfelder et al., 2000).

Although studied extensively in pigs, and to a lesser extent cattle, the validity of QBA to assess sheep welfare has been less well studied (Table 1.13). To the authors' knowledge, only eight studies (excluding those chapters presented within this thesis that are published; $n = 4$) attempt to validate QBA in sheep (Table 1.14), half of which address the welfare of sheep during transport (Wickham et al., 2012; Fleming et al., 2015; Wickham et al., 2015; Collins et al., 2018). Importantly, Phythian et al. (2016) have demonstrated not just validity but on-farm feasibility, reporting meaningful associations between observer QBA scores and physical health measures including lameness, dull demeanour and breech soiling in sheep kept under commercial conditions. However, there is a clear need for further studies to evaluate QBA in sheep for practical application. There is thorough evidence that observer assessments of sheep behavioural expression using the QBA methodology are reliable (see; Table 1.14), meaning that not only do different observers come to the same conclusion (inter-observer reliability or consensus) but that the same observers could repeat their assessments (intra-observer reliability), both of which measures are essential for the assessment of animals under field or commercial conditions (Tuyttens et al., 2014).

In terms of practical on-farm application, QBA is well suited, being quick, easy to implement, non-invasive, and can be done on individual animals or groups of animals from either direct observation or video footage (Fleming et al., 2016). Indeed, meaningful assessments of sheep have been achieved on video footage as short as 60 sec (see; Table 1.14). By contrast, under commercial conditions, Knierim and Winckler (2009) determined that it would take an estimated 30 min to assess up to 200 cattle. It is perhaps for these reasons, alongside the fact that QBA can indicate positive welfare states in animals, that QBA has been incorporated in formal welfare protocols such as the Welfare Quality® protocols for cattle (Welfare Quality®, 2009a), pigs (Welfare Quality®, 2009b) and poultry (Welfare Quality®, 2009c), and the AWIN protocols for donkeys (AWIN, 2015a), goats (AWIN, 2015b), horses (AWIN, 2015c) and sheep (AWIN, 2015d). Furthermore, in many of these species, QBA has been evaluated against the other measures also incorporated in these protocols, including in cattle (e.g. Andreasen et al., 2013), pigs (e.g. Temple et al., 2011; Otten, 2013; Munsterhjelm et al., 2015), chickens (e.g. Muri et al., 2019), goats (e.g. Battini et al., 2016; Battini et al., 2018) and donkeys (e.g. Dai et al., 2016; Minero et al., 2016). Overall, it is possible for QBA to be a useful and feasible tool to assist in the practical assessment of sheep welfare.

Table 1.14. Summary of studies that investigate the validity and reliability of qualitative behavioural assessment (QBA) to inform welfare in sheep. The approach to QBA, method of data collection and observation method, length of time the animals were observed during the assessment and the number of observers used in studies is also presented. The experimental chapters within this thesis that are also published are not presented here.

Approach to QBA*	Data collection method	Length of observation	Observation method	Number of observers	Validity^	Reliability£		References
						Inter-observer	Intra-observer	
FCP	Video footage	20 – 60 sec	Individual	63	T	P	−	Wickham et al. (2012)
FCP	Video footage	1 min	Individual	13	t	P	−	Cockram et al. (2012)
FCP	Video footage	2 min	Individual	11	T	P	−	Stockman et al. (2013b)
FL	Video footage	1 min	Individual and group	13	−	T	−	Phythian et al. (2013a)
FCP	Video footage	Study 1: 20 – 60 sec Study 2: 2 min	Individual	Study 1: 63 Study 2: 32	Study 1: T Study 2€: −	Study 1: P Study 2: T	−	Fleming et al. (2015)
FCP	Video footage	20 – 60 sec	Individual	57	T	P	−	Wickham et al. (2015)
FL	Direct observation	5 min	Group	1	T	P	T	Phythian et al. (2016)
FCP	Video footage	1 min	Individual	10	T	P	−	Serrapica et al. (2017)
FL	Study 1: Video footage Study 2: Direct observation	2 min	Group	Study 1: 8 Study 2: 3	Study 1: − Study 2: −	Study 1: T Study 2: T¥	−	Muri and Stubsjøen (2017)
FCP	Video footage	1 min	Individual	Study 1: 26 Study 2: 20	Study 1: T Study 2: T	Study 1: P Study 2: P	−	Collins et al. (2018)
FL	Video footage	2 min	Group	6	−	T	T	Diaz-Lundahl et al. (2019)

* Approach to qualitative behavioural assessment (QBA): FL = Fixed list; and FCP = Free-choice profiling.
^ Validity: T = significant associations between assessment criterion; t = tested not significant; and − = not tested.
£ Reliability: T = additional measures of reliability were conducted, and significant agreement was recorded between observers i.e. Kendall coefficient of concordance (*W*) or Spearman rank order correlation (r_S) where $P < 0.05$; P = no additional measures of reliability were conducted but observers reached significant consensus in their assessment as indicated by a significant Procrustes test statistic where $P < 0.001$; t = tested but not significant; and − = not tested.
€ This study investigated the sensitivity of visual or verbal information on the observers QBA scores of the same sheep. Spearman rank order correlations were conducted between the observer QBA scores given to same animals from two different groups (information and no information).
¥ Achieved moderate agreement according to Kendall coefficient of concordance but significance of *W* score was not available.

1.4.2.3.3 General considerations on the assessment of behaviour to inform welfare

Behavioural measures can provide useful information concerning whether sheep are having difficulty coping with challenges (physical and psychological) and they have the advantage over many physiological measures in that they are i) non-invasive, ii) (generally) non-intrusive, iii) relatively simple to record, iv) do not require laboratory testing and v) provide an assessment of affective state. However, like physiological measures, the use of behavioural measures to assess sheep welfare is challenging. There are not just one or two primary behaviours producers can use to monitor to inform welfare; rather, numerous measures are available (as illustrated in the preceding sections). Thus, the time required to conduct meaningful behavioural assessments is a major limitation (Goddard, 2011; Barrell, 2019). Even if resources (time, labour, infrastructure, and finance) allowed for numerous behaviours to be collected routinely (i.e. at key production times) or remotely (e.g. video camera technology), even automatically (e.g. biosensors), we cannot afford to record numerous behaviours and then investigate each and every incidence of altered behaviour. There are also problems with interpretation; with determining if and how the behavioural changes observed actually relate to welfare (Dawkins, 1980). At this point, it is also important to recognise the complexity of animal behaviour, and that the assessment and interpretation of behaviour to inform welfare is not always straightforward. Particularly since behaviour may serve more than one function (Fraser and Broom, 1990; Broom, 2010), and because individuals may adopt a different behavioural response to the same challenge (Broom and Johnson, 2019b). Thus, a better understanding of the behavioural responses of sheep to common welfare issues is required. Furthermore, the study of behaviour to inform affective state should always be done with care (Kremer et al., 2020).

1.5 GENERAL AIMS AND HYPOTHESES

It is reasonable to say that it is not simple to measure welfare in sheep, particularly under commercial conditions. Even though numerous measures could aid sheep producers monitor and assess the welfare of their stock, there are no obvious primary measures that provide a comprehensive assessment of animal welfare to inform management decisions. Furthermore, many of those measures available were developed for clinical or scientific purposes, or for the evaluation of management practices (e.g. different husbandry systems, housing, or procedures), rather than to assess the actual welfare state of an animal. Consequently, outside of a few measures (e.g. BCS and locomotion scoring to identify lameness), many measures have not been well explored for this purpose, particularly under practical conditions where they are most needed. Although these few measures are our only means to gain insight into the welfare of sheep under commercial conditions, each has issues that may limit their usefulness and appropriateness. Furthermore, it is often difficult to weigh and integrate multiple measures to get a clear picture of animal welfare (Broom and Johnson, 1993; Hewson, 2003; Fraser, 2008), and then to identify the point at which changes in those recorded behaviours indicate compromised welfare (i.e. thresholds). Thus, it is critical to identify a small number of easy to use and transparent, yet meaningful measures, which could be integrated into a simple tool to facilitate the recognition of sheep with compromised welfare and to inform management decisions.

While it appears that behaviour is suited best for sheep welfare assessment on-farm, being more easily obtainable than physiological measures under commercial conditions, there are still issues with interpretation and the combination of these measures to come to a complete picture of the animal's welfare state. For this reason, QBA as an assessment of 'whole' animal welfare, is promising. The general aim of this thesis was to explore and evaluate the QBA methodology to assess the welfare of sheep under commercial situations and to examine the relationship between quantitative and qualitative behaviours, and clinical observations, of animals that faced several common welfare issues that are important to the Australian sheep industry: flystrike, gastrointestinal parasites, lameness, inappetence, and pain caused by husbandry procedures, as well as the perceived positive role of acclimation to human presence.

CHAPTER 2 GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

The general material and methods used in the studies that contribute this thesis are described in this chapter. Specific methods used in each study are still described in the relevant chapters (Chapters 3 – 6).

2.2 QUALITATIVE BEHAVIOURAL ASSESSMENT (QBA)

2.2.1 Recruitment of observers

The recruitment of observers for QBA in these four studies was achieved through advertising. Specifically, advertisements were in the form of emails, flyers around the University campus, and on social media. All persons that responded to these advertisements were accepted into the study, with total of 120 observers participating across the four studies that make up this thesis.

2.2.2 Free-choice profiling (FCP) approach

In each study, the recruited observers were given a detailed introduction to QBA and its concepts, followed by specific instructions on completing the QBA scoring sessions. This introduction involved explaining the concepts of QBA, specifically highlighting the differences between this approach and more traditional quantitative measures. Examples were provided and discussed, and in each case confirmation that each person understood the concepts of QBA was obtained. Once observers were clear on the concepts of QBA, they were given instructions on how to complete the assessment itself. Since the FCP approach to QBA requires attendance to two different session; a term generation session followed by an assessment or quantification session, the specific instruction for these different sessions were presented at the beginning of the relevant session. It is important to note that in this introduction, the observers were not given any details on the animals or the experimental treatments.

2.2.2.1 Term generation session

The purpose of the term generation session was to have observers create their own unique lists of descriptive terms which they would then use to score the assessment animals in the quantification session. To generate their lists of terms, observers were shown a series of video clips that depicted sheep performing a wide range of behavioural expressions, experimental and environmental conditions, to allow observers to describe as many aspects of the sheep's expressive repertoire as possible. The number of video clips the observer watched in this session differed between studies (range: 9 – 12). Briefly, in these videos observers were shown examples of both healthy and unhealthy sheep, where video clips included sheep that were in isolation or in groups, within a test arena, yard or in a paddock. After watching each video clip, observers were given 2 min to write down terms they thought described the animal's behavioural expression. There was no limit imposed on the number of descriptive terms an observer could generate, but terms needed to describe how the animal behaved (e.g. nervous, relaxed), rather than what the animal was doing (i.e. physical descriptions of the animal such as vocalising, chewing, tail flicking). Once each observer had finished watching the clips, their lists were edited to remove terms that described actions, and terms that were in the negative form were transformed to the positive (e.g. 'unhappy' became 'happy'). This was done for three reasons, i) to compensate for the apparent bias in the terms generated towards a negative meaning (e.g. approximately 60% of the terms generated by observers in the study that is described in Chapter 3 could be classified as negative, with the remaining 40% positive or neutral), ii) for ease of scoring, and iii) to ensure consistency between observers. In cases where the observer generated a term with no positive form (e.g. uneasy) or where the observer felt that the positive form of the term was not adequate on its own, the original term was retained. At this point, it was confirmed that the observer was happy with their unique lists and the session was concluded.

2.2.2.2 Quantification session

In the quantification session, observers used their own unique lists of terms to score the assessment animals in each study. For observers to score sheep, each unique descriptive term they generated in the previous session was attached to a visual analogue scale (minimum to maximum expression of that term) in an electronic worksheet (Microsoft Excel 2003, North Ryde, NSW, Australia). These terms were randomly arranged within this worksheet. At the beginning of this session, the observers were given detailed instructions on how to use their lists to assess the focal sheep. Specifically, observers were instructed to score each animal's expression using the visual analogue scale by placing an 'x' at the appropriate point between the two extremes of the scale bar, where minimum (= 0) reflected the absence of expression of that particular descriptive term, and maximum (= 100) indicated the animal could not show an expression more strongly. The distance between the minimum-point and their mark on the scale as reflected the intensity of each animal's expression on that term. The observers were also given clear instructions to score each assessment clip on all descriptive terms using the attached visual analogue scale, so that each animal was assessed on all terms. The Observers were provided with individual workstations and worked at their own pace through the assessment clips. At the end, the scoring sheets were checked to ensure no terms were missed.

2.3 STATISTICAL ANALYSIS (QBA)

The statistical analysis for QBA is considered quite complex, however a basic description of the analyses in presented here. The individual clip scores, that is the distance from the start of the visual analogue scale (0 – 100) to where the observer had marked an 'x', for each observer in a study are submitted for analysis by means of Generalised Procrustes Analysis (GPA) (GenStat 2008-2018, VSN International, Hemel Hempstead, UK; Wemelsfelder et al. (2000)). For a detailed description of GPA analysis and output interpretation procedures see Wemelsfelder and colleagues (2000; 2001).

Briefly, GPA is a multivariate technique that identifies underlying patterns in observer assessments (i.e. descriptive terms of the animal's behavioural expression) and calculates the level of consensus between observer assessments of the individual animals. The statistical process whereby this best-fit pattern, termed the consensus profile, is identified takes place independently of the meaning of descriptive terms used by observers. The percentage of variation between observers (in their assessment of individual sheep) that is explained by the consensus is captured as the Procrustes statistic. The statistical performance of the consensus profile above chance is calculated by comparing (using a one-sample t-test) the Procrustes statistic to the mean of a simulated distribution of 100 Procrustes statistics generated through 100 iterations of the analysis, where the data is randomised in a different permutation each time. Significance values in that test of $P < 0.001$ or better can be taken as evidence that the consensus profile was not a methodological artefact and represents a common pattern identified by observers.

The consensus profile is then simplified to a smaller number of dimensions (two – three), explaining the majority of variation between observed animals, by Principal Component Analysis (PCA). To allow semantic interpretation of these main dimensions, the individual observer's terms with the strongest correlation coefficients with the consensus dimension scores were identified. This process was entirely post hoc to the computation of the consensus profile.

At the end of these analyses, each assessment clip (animal) received a score on the main GPA consensus dimensions. It is these scores that analysed for treatment differences. In all cases, these scores were tested for normality, and where required non-parametric analyses were used. The specific statistical analyses performed in each study from this point are detailed in the relevant statistical analysis sections of each experimental chapter (Chapters 3 – 6).

CHAPTER 3 REMOTE IDENTIFICATION OF SHEEP WITH FLYSTRIKE USING BEHAVIOURAL OBSERVATIONS

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3.1 ABSTRACT

Flystrike is a major problem affecting sheep in Australia. Identification of 'flystruck' individuals is crucial for treatment, but requires labour-intensive physical examination. As the industry moves toward more low-input systems, there is a need for remote methods to identify flystruck individuals. The aim of this study was to investigate the behaviour of sheep with breech flystrike within a paddock setting. Video footage of sixteen Merino sheep, eight later confirmed with flystrike and eight without, was collected as they moved freely within the paddock with conspecifics. Quantitative behavioural measurements and a qualitative behavioural assessment (QBA) were conducted and compared to their breech conditions (i.e. faecal/urine staining, flystrike severity). Both qualitative and quantitative assessments indicated behavioural differences between flystruck and non-flystruck animals. Flystruck sheep had a behavioural profile characterised by restless behaviour, abnormal postures and reduced grazing time ($P < 0.05$). Furthermore, flystruck sheep were scored to have a more *'exhausted/irritated'* demeanour using QBA ($P < 0.05$). The behavioural responses also corresponded to the flystrike severity scores and condition of the breech area. We conclude that remotely-assessed behaviour of flystruck sheep diverges markedly from non-flystruck sheep, and thus could be a low-input method for identifying and treating affected animals.

3.2 INTRODUCTION

Flystrike, or cutaneous myiasis, is a major health and welfare problem within the Australian sheep industry. The disease of flystrike is caused by the chemical and mechanical effects of blowfly (subfamily Calliphoridae) larvae (maggots) as they feed on the host's dermal tissue and inflammatory exudates (Tellam and Bowles, 1997; Wall, 2012; Mauldin and Peters-Kennedy, 2016; Anstead et al., 2017). Onset of disease is rapid (Gibson et al., 1984; Horton et al., 2018), and is characterised by cutaneous lesions, pyrexia (fever), inflammation and the severe irritation of the skin (Plant, 2006; Mauldin and Peters-Kennedy, 2016). Infested sheep can experience reductions in feed intake, body weight, wool production and lamb losses (Broadmeadow et al., 1984; Horton et al., 2018). More important, however, is the risk of death from bacterial and/or systemic toxaemia in severe or untreated cases (Broadmeadow et al., 1984; Wardhaugh and Morton, 1990). It stands to reason that, when not properly managed, flystrike represents a debilitating disease that raises significant welfare concerns.

Presently, to minimise stock susceptibility, sheep producers rely on strategies that incorporate both preventative treatments such as drenching, spraying with chemical treatments, crutching and shearing, and the management of factors that predispose animals to flystrike, such as gastrointestinal parasites and diarrhoea (Phillips, 2009; Sandeman et al., 2014). However, such management strategies are often labour-intensive and costly. Moreover, due to recent opposition to mulesing (removing folds of skin from the breech area to reduce risk of flystrike), producers have been encouraged to reduce their dependence on this surgical practice; thus there is heavy reliance on the use of insecticides to manage flystrike (Sandeman et al., 2014). These management strategies do not appear to offer long-term or reliable protection against severe outbreaks of flystrike (Wardhaugh et al., 2007), however, and frequent physical examination of penned animals is fundamental, particularly during severe flystrike seasons typically seen during the Spring months. Such monitoring imposes significant costs to producers in terms of time and labour. The development of simple, animal-based, within-paddock (remote) indicators would give producers a tool to aid decision making regarding the management of flystrike. Currently, no formal protocols are available that target remote visual identification of flystruck sheep.

Behaviour plays an important role in the diagnosis and early detection of health issues and diseases (Dawkins, 2004; Gougoulis et al., 2010). There is some evidence to suggest that flystruck sheep display behaviours indicative of agitation (Anderson et al., 1988). However, to date, there have been no studies that focus on the application of behavioural assessments for the early identification of flystrike in sheep. Animal behaviour is complex and dynamic, and as such, it stands to reason that assessments should be comprehensive and endeavour to reflect the state of the whole animal. Qualitative Behavioural Assessment (QBA) has been proposed as one such ‘whole-animal’ measure, with observers providing assessments of expressive behaviour (body language) through the integration and summary of details of behaviour, posture and movement, and context (Wemelsfelder et al., 2001; Wemelsfelder and Lawrence, 2001; Fleming et al., 2016). The aim of this study was to investigate the behaviour, using QBA and quantitative measures, of sheep with and without breech-strike within a paddock setting. Differences in QBA scores for sheep with flystrike may be useful as an indicator for further management options to improve their health and welfare.

3.3 MATERIALS AND METHODS

This experiment was approved by the Animal and Human Ethics Committees at Murdoch University (R2598/13; N2779/15; O2780/15; 2008/021) and the Animal Ethics Committee of the Department of Agriculture and Food Western Australia (AEC 1-14-02) to ensure compliance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, the Australian Code for the Responsible Conduct of Research 2007, and the National Statement on Ethical Conduct in Human Research, 2007.

3.3.1 Animals and experimental design

The behaviour of 16 mature Merino ewes categorised into two groups based on physical examination as flystruck (n = 8) and non-flystruck (n = 8), were assessed from video footage collected in the paddock. Video footage was collected from ewes within three paddocks (200 – 300 individuals) during routine inspections for flystrike over a 7-day period. During the inspections, a dedicated and trained member of the research team was responsible for the remote (~ 50 m distance) visual identification of suspected Flystruck and non-flystruck animals for filming and subsequent physical examination. Sheep positively identified as being flystruck by trained personnel, i.e. by the presence of maggots and/or cutaneous lesions, were treated immediately after filming, with the wool around the flystrike area clipped and treated with a short-acting, kill-on-contact insecticide, Extinosad® (Elanco Animal Health, Australia) as per manufacturer's recommendations. Those sheep that were categorised as non-flystruck at visual inspection were not treated. Weather conditions for the duration of this study were consistent and there were no apparent unusual climactic events that could bias behavioural recording. Although multiple types of flystrike were recorded, the behavioural assessment of flystruck sheep in the present study was confined to breech flystrike (seven animals) or breech and rump flystrike (one animal), where breech is defined as the area around the tail.

3.3.2 Breech soiling assessment

Indicators of breech soiling: dag, dag moisture and urine stain scores were collected by trained personnel at the time of physical examination in the present study. Breech soiling with faeces (dags), dag moisture content and urine stain were scored using a five-point scoring method (AWI, 2007; Greeff et al., 2014), in which 1 denoted no dags, dry dags and no stain; and 5 was the highest score for each trait, indicating extensive dags, very wet dags and extensive urine staining, respectively. With regard to urine stain, severity was defined by the diameter of the affected area (Scholtz et al., 2010) and scored on a five-point scale between 1 (mild) and 5 (severe), whereby 1 = 1–5 cm^2; 2 = 5–10cm^2; 3 = 10–15cm^2; 4 = 15–20cm^2; and 5 > 20cm^2.

3.3.3 Behaviour

The behaviour of flystruck and non-flystruck sheep were analysed from footage collected in the paddock using a hand-held video camera (Panasonic HC-W570M: Panasonic Corporation, Kadoma, Japan) in a fixed position approx. 50 m from the mob. To avoid sampling bias, filming of focal animals within the mob for behavioural assessment occurred 10 s after the trained personnel visually identified suspected flystruck and no-flystruck sheep. Although flystrike may occur without faecal soiling of the breech region, to avoid visual discrimination of the flystruck status of animals by observers, where possible footage was predominately captured from the front and side of animals. After physical inspection and, if required, treatment, sheep were returned to the group and allowed to settle for 10 – 20 min to minimise potential effects of disruption before stock were again surveyed for filming. Footage collected on-farm was subsequently reviewed and edited to depict sheep for analysis. Resulting video clips were approximately 20 s duration (20.5 ± 0.6 s). To ensure that the results of the quantitative behavioural scoring were comparable with those from the QBA analysis, both quantitative and qualitative behavioural assessments were conducted on these video clips.

3.3.3.1 Quantitative behaviour scoring

Three experienced observers blinded to the treatment groups scored footage for the 16 sheep for the incidence of abnormal behaviour (**Error! Reference source not found.**a), and the number of sheep engaged in each of these activities was calculated for each treatment group. A score of 'restlessness' was calculated for each animal as the total number of interruptions or changes to the predominant behaviour (**Error! Reference source not found.**b). The percentage of total time each individual animal spent walking, grazing, total standing, and standing with an abnormal posture was also recorded (**Error! Reference source not found.**c); every animal was considered to be engaged in one of these activities for the duration of observation.

Table 3.1. Description of behaviour used to score sheep from 20 s video clips: (a) abnormal behaviour, (b) restlessness, and (c) percentage of time spent walking, grazing and standing.

Behaviour	Description
a) Abnormal behaviour (count of sheep per treatment group showing each of these activities at least once over the clip duration)	
Kicking	Either front or hind limb was raised and forcefully strikes the ground or is moved backwards or forwards without moving other limbs.
Tail wagging	Rapid and repetitive side-to-side tail movements separated from another tail wagging event by at least 2 sec.
Head turning	Turning head beyond the shoulder.
Biting rump region	Turing head beyond the shoulder and sheep actively biting rump area.
b) Score of 'restlessness'	
Restlessness	The number of interruptions or changes to the predominant behaviour (walking, grazing or standing) of the sheep for the combined (abnormal) behaviours of kicking, head shake, head turning, biting rump region and tail wagging.
c) Percentage of time spent (% of total time observed)	
Walking	Moving forward in a four beat motion for 2 s or more with head orientated in direction of movement.
Grazing	Actively chewing pasture. Head may be lowered towards ground or raised if chewing.
Total standing	Standing stationary on four legs, without jaw movement indicative of chewing. Includes normal standing with head and neck in normal or neutral position, and abnormal standing (see below) restricted to when animal was not actively grazing.
Abnormal standing	Abnormal head and neck posture when standing stationary includes standing with head lowered (below withers) and hunched back, head orientated towards the side, neck extended and head in low position or head turned towards rump region. Restricted to when animal was not actively grazing.

3.3.3.2 Qualitative behavioural assessment (QBA)

A total of 26 observers were recruited from Murdoch University staff and students (17 female, 8 male and 1 unidentified) to assess the videos using the free-choice profiling (FCP) methodology. Observers were blind to the study objectives and treatments investigated at the time of assessment. All but two observers were naïve to the QBA methodology, with the two observers indicating that their previous experience with the QBA methodology was used to assess non-sheep species. Removal of these two observers did not alter the overall significance of the consensus and all observers fell within the 95% confidence interval, indicating the high level of agreement between observers in their use of descriptive terms to quantify the behavioural expression of sheep. Thus, all 26 observers were retained in the consensus.

Observers completed a short survey regarding their past experiences with sheep and other domestic livestock species upon initial contact. Of the 26 observers, 16 (61.5%) were classified as completely inexperienced with sheep, indicating that they had never spent time working with sheep. Nine (34.6%) had limited experience working with sheep. The remaining observer (3.8%) indicated that they were experienced, having worked with sheep for more than a year.

Observers were required to attend two sessions; a term generation session followed by the quantification session. Observers were given detailed instructions on completing the QBA scoring sessions but were not given any details about the animals or the experimental treatments.

3.3.3.2.1 Session 1 – Term generation and training

Observers were shown 10 video clips (average 26 ± 5 s duration), which were not used in the assessment session, that depicted sheep performing a wide range of behavioural expressions, experimental and environmental conditions, to allow observers to describe as many aspects of the sheep's expressive repertoire as possible. Briefly, observers were shown examples of both healthy (n = 5) and unhealthy sheep (n = 5), where video clips included sheep that were in isolation or in groups, within a test arena or in paddock, and/or were flystruck, lame, inappetent, had high or low faecal egg counts, or were healthy (footage was collected over a range of treatments, including those used for other QBA studies; Grant et al., 2018). After watching each video clip, observers were given 2 min to write down terms they thought described the animal's behavioural expression. There was no limit imposed on the number of descriptive terms an observer could generate, but terms needed to describe how the animal behaved (e.g. nervous, relaxed), rather than what the animal was doing (i.e. physical descriptions of the animal such as vocalising, chewing, tail flicking). Subsequent editing of the descriptive terms was carried out to remove terms that described actions, and terms that were in the negative form were transformed to the positive for ease of scoring and to ensure consistency between observers (e.g. *'unhappy'* became *'happy'*). The result was a unique list of descriptive terms for each of the 26 observers to be used for quantification in Session 2. For observers to score sheep, each unique descriptive term was attached to a visual analogue scale (minimum to maximum expression of that term) in an electronic worksheet (Microsoft Excel 2003, North Ryde, NSW, Australia) and for each observer, the list of terms were randomly arranged within this worksheet.

3.3.3.2.2 Session 2 – Quantification

Observers viewed and scored video clips of the 16 assessment sheep using their own unique lists of descriptive terms. Observers were instructed to score each animal's expression using the visual analogue scale by placing an 'x' at the appropriate point between the two extremes of the scale bar, where minimum (= 0) reflected the absence of expression of that particular descriptive term, and maximum (= 100) indicated the animal could not show an expression more strongly. The distance between the minimum-point and their mark on the scale as reflected the intensity of each animal's expression on that term.

3.3.4 Statistical analysis

All statistical analyses were carried out using GenStat 18 (Genstat 2018, VSN International, Hemel Hempstead, Hertfordshire, UK) and Excel for Windows 2016 (Microsoft Inc, Redmond, WA, USA). All data was tested for normality (Shapiro-Wilk tests), and where required, non-parametric analyses were used.

3.3.4.1 Quantitative behaviour scoring

Inter-observer reliability and the concordance between the three observers were evaluated by Kendall's coefficient of concordance. Chi-square tests were used to compare count data between treatment groups (flystruck or non-flystruck animals) for the four abnormal behaviours recorded. A Students *t*-test was used to investigate differences in total restlessness scores between groups. For the behavioural time-budget categories, Mann-Whitney *U* tests were used to identify differences between treatment groups. In all cases, the standing observed in assessment animals was classified as Abnormal, consequently, Total Standing was not reported herein.

3.3.4.2 Qualitative behavioural assessment

For QBA, the distance from the start of the visual analogue scale to where the observer had made a mark for each term was measured (where minimum = 0 and maximum = 100) and these data were analysed by means of Generalised Procrustes Analysis (GPA) (Genstat 2008, VSN International, Hemel Hempsteat, UK; Wemelsfelder et al. (2000)). For a detailed description of GPA analysis and output interpretation procedures see Wemelsfelder and colleagues (2000; 2001).

Briefly, GPA is a multivariate technique that identifies underlying patterns in observer assessments (i.e. descriptive terms of the animal's behavioural expression) and calculates the level of consensus between observer assessments of the individual animals. The statistical process whereby this best-fit pattern, termed the consensus profile, is identified takes place independently of the meaning of descriptive terms used by observers. The percentage of variation between observers (in their assessment of individual sheep) that is explained by the consensus is captured as the Procrustes statistic. The statistical performance of the consensus profile above chance is calculated by comparing (using a one-sample *t*-test) the Procrustes statistic to the mean of a simulated distribution of 100 Procrustes statistics generated through 100 iterations of the analysis, where the data is randomised in a different permutation each time. Significance values in that test of $P < 0.001$ or better can be taken as evidence that the consensus profile was not a methodological artefact and represents a common pattern identified by observers. As an additional measure of inter-observer reliability between observers, scores for each individual clip were correlated using Kendall's coefficient of concordance *W*.

The consensus profile is then simplified to a smaller number of dimensions (in this case, two), explaining the majority of variation between observed animals, by Principal Component Analysis (PCA). To allow semantic interpretation of these main dimensions, the individual observer's terms with the strongest correlation coefficients with the consensus dimension scores were identified. This process was entirely post hoc to the computation of the consensus profile.

Students *t*-tests were used to test for a treatment effect (flystruck or non-flystruck animals) on the average scores for each of the sheep on these two GPA dimensions. Demographic information was presented to demonstrate the level of experience QBA observers had with sheep but were not statistically analysed.

3.3.4.3 Associations between breech soiling assessment, behaviour scoring, and QBA

A PCA based on standardised variables was used to investigate the relationship between the behavioural, both quantitative and qualitative, and breech soiling data. Variables were interpreted according to their loadings on the most important components (PCA 1 and 2), and variables with high loadings on the same component can be grouped. Such groupings indicate which of the variables recorded were most closely related and those that had the greatest association with the treatment groups. This served to determine whether the GPA dimensions were associated with known behavioural parameters of stress and physical indicators of flystrike and poor welfare. The PCA (correlation matrix, no rotation) was performed by analysing individual animal data on all 14 variables (8 behavioural parameters, 4 breech soiling measures and individual animal scores on QBA dimension 1 and 2). A urine stain score was missing for one animal from the flystruck group, thus the corresponding animal was not submitted to the PCA. Spearman rank order correlations were also employed to examine the association between the collected parameters.

3.4 RESULTS

3.4.1 Breech soiling assessment

Average dag scores of flystruck animals at time of treatment was 3.25 ± 0.37 (mean ± S.E.M..; range: 2–5), indicating moderate to high episodes of diarrhoea. Average dag moisture content was moderate with sheep recording scores of 2.00 ± 0.20 (mean ± S.E.M.; range: 1–3). Urine stain scores were moderate across the assessed animals, averaging 2.14 ± 0.24 (mean ± S.E.M.; range: 1–3). Severity of flystrike was not very high, with five animals (62.5%) having a severity score of 2 (size of flystrike: 5–10 cm^2) and the remaining three animals (37.5%) receiving moderate scores of 3 (size of flystrike: 10–15 cm^2). Although none of the recorded flystrikes were classified as mild (being less than 5 cm^2), there were no scores indicative of severe flystrike.

3.4.2 Quantitative behaviour scoring

Table 3.2 shows the Kendall's coefficient of concordance (*W*) for the degree of agreement between the three observers who analysed the behaviour of sheep from video footage. These results show that for each behaviour recorded, the observers showed significant agreement on the ranking of the focal sheep. Given this result, in subsequent analysis of quantitative behaviour, we considered only the data of one trained observer scoring the 16 sheep observed.

Table 3.2. The degree of agreement between observers on video-based assessment of sheep behaviour.

Behavioural parameters	Kendall *W*	*P*
a) Abnormal behaviour		
Kicking	0.77	0.003
Tail wagging	0.84	< 0.001
Head turn	0.82	0.001
Biting rump region	1.00	< 0.001
b) Restlessness		
	0.89	< 0.001
c) Percentage of time spent		
Walking	0.94	< 0.001
Grazing	0.90	< 0.001
Abnormal standing	0.84	< 0.001

There was a significant treatment effect in the number of animals exhibiting abnormal behaviour (Table 3.3a). More of the flystruck sheep turned their head (P = 0.005) and actively bit their rump region (P = 0.002) compared to the non-flystruck animals (none performed either of these behaviours). In addition, although kicking and tail wagging was also observed in the non-struck animals, more of the flystruck sheep were observed to display these behaviours compared to the non-flystruck animals (P = 0.015 and P = 0.011, respectively).

Overall restlessness also showed a significant treatment effect, with flystruck animals recording 3.9 fold increase in interruptions to normal grazing behaviour (including kicking, tail wagging, head shaking, head turning and biting rump) compared to non-flystruck animals (F_{14} = -4.77, P < 0.001; Table 3.3b).

There were also significant treatment effects for the percentage of time sheep spent walking, grazing and standing, with flystruck sheep spending less time grazing (*U* = 4, *P* = 0.002) and more time standing with abnormal posture (*U* = 3, *P* = 0.001) than non-flystruck animals (Table 3.3c). There was no significant treatment effect for the proportion of time the sheep spent walking (Table 3.3c).

Table 3.3. Comparison of behavioural scoring for sheep in Flystruck and Non-flystruck treatment groups taken from video clips of 20 s duration.

Behavioural parameters	**Raw value**	
	Flytruck (*n* = 8)	**Non-flystruck (*n* = 8)**
a) Abnormal behaviour (count of sheep per treatment group)		
Kicking	3 (37.5%) a	1 (12.5%) b
Tail wagging	5 (62.5%) a	1 (12.5%) b
Head turn	4 (50.0%) a	0 (0.0%) b
Biting rump region	5 (62.5%) a	0 (0.0%) b
b) Restlessness (no. instances)		
	4.89 ± 0.55 a	1.25 ± 0.53 b
c) Percentage of time spent (% of total time observed)		
Walking	0.17 ± 0.08 a	0.15 ± 0.06 a
Grazing	0.09 ± 0.09 a	0.74 ± 0.07 b
Abnormal standing	0.73 ± 0.11 a	0.12 ± 0.07 b

Values are percentages of sheep per treatment exhibiting each behaviour or mean ± S.E.M.. For abnormal behaviours, different letters indicate significant differences between treatment groups using Chi-Squared (at P < 0.05), whereas different letters indicate significant differences using Students t-test and Mann-Whitney U-tests (at P < 0.05) for restlessness and percentage of time spent walking, grazing and standing abnormally, respectively.

3.4.3 Qualitative behavioural assessment

The 26 observers generated a total of 66 unique terms to describe the sheep they were shown (average 13.5 ± 3.4 terms per observer; range 8 - 21) using the FCP methodology. The GPA consensus profile explained 51.4% of the variation between observer scores of sheep and this differed significantly from the mean randomised profile ($t99$ = 34.89, *P* < 0.001). Two main dimensions of behavioural expression were identified, explaining a total of 67.7% of the overall variation in scores attributed to individual sheep (Table 3.4). Observers showed a moderate to good level of agreement for the two main dimensions, with Kendall's *W* values of 0.56 and 0.66, respectively (Table 3.4).

The word charts for the observers appeared to be semantically consistent across the observers. Terms with the strongest positive and negative loadings within each of the GPA dimension are shown in Table 3.4. Low values for GPA dimension 1 were associated with descriptive terms such as *'exhausted'* and *'irritated'*, and high values with terms such as *'positively occupied'* and *'assured'*. For GPA dimension 2, low values were associated with descriptive terms such as *'indecisive'* and *'depressed'*, and high values with terms such as *'inquisitive'* and *'collected'*.

Flystruck sheep were scored as significantly more *'exhausted/irritated'* on GPA dimension 1 compared with non-flystruck sheep, which were scored as more *'positively occupied/assured'* (Figure 3.1a) (t14 = 4.38, P < 0.001). There were no significant differences between the two treatment groups on GPA dimension 2 (t14 = -0.97, P = 0.35).

Table 3.4. Terms used by observers using the Free-choice Profiling (FCP) method of Qualitative Behavioural Assessment (QBA) to describe the behavioural expression of sheep filmed in paddock.

GPA dimension (% of variation explained) Kendall's *W*	**Descriptive terms†**	
	Low values	**High values**
GPA 1 (58.1%) 0.66***	Exhausted (1), irritated (8), tense (1), agitated (7), frustrated (4), distressed (8), alarmed (1).	Positively occupied (1), assured (1), unhurried (1), active (1), content (12), relaxed (21), settled (2), happy (18).
GPA 2 (9.6%) 0.56***	Indecisive (1), depressed (2), afraid (1), insecure (2).	Inquisitive (4), collected (1), dull 1), interested (2), lively (2), aggressive (3), alarmed (1), unhurried (1), joyful (2), settled (2), cautious (8).

*** P < 0.001.
† Terms that had strong loadings with the Generalised Procrustes Analysis (GPA) dimensions are listed. Terms shown have loadings of > 0.6 (high values) and < -0.6 (low values) for GPA dimension 1, and >0.3 (high values) and < -0.3 (low values) for GPA dimension 2. Numbers in parentheses represent the number of observers that generated and subsequently used that word to assess sheep expressive behaviour.
The three highest weighting terms for each GPA dimension were selected for the purpose of labelling the GPA dimensions and describing the dimensions in relation to experimental group (Figure 1).

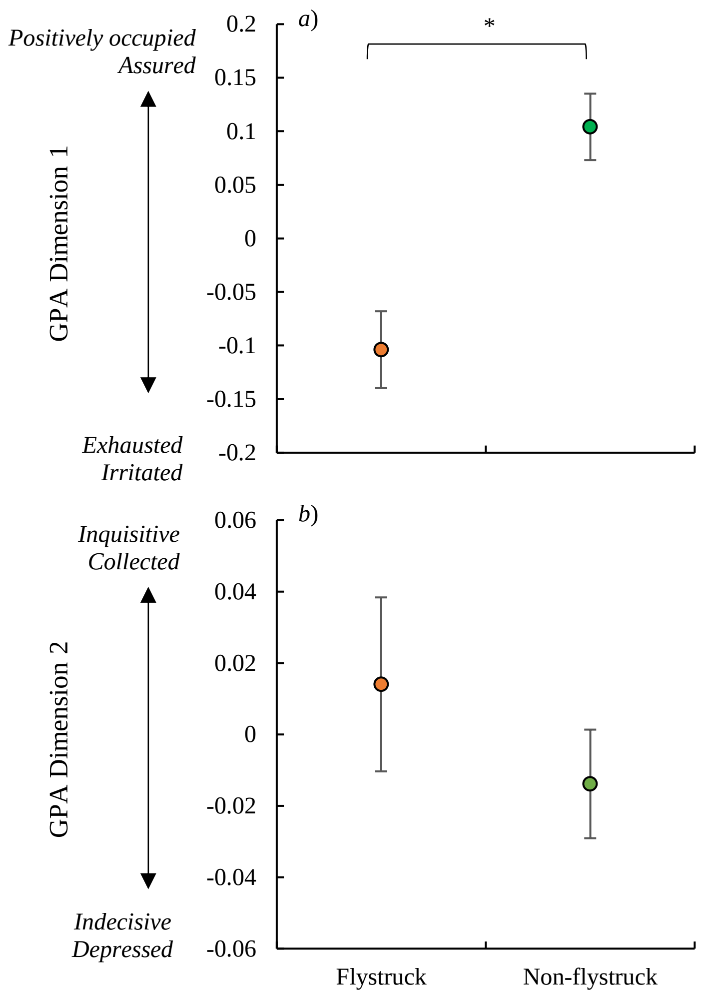

Figure 3.1. Effects of the treatment groups: Flystruck (orange marker; *n* = 8) and Non-flystruck (green marker; *n* = 8) on General Procrustes Analysis (GPA) scores for Qualitative Behavioural Assessment (QBA) dimension (*a*) 1; and (*b*) 2 assessed from video footage taken of sheep in paddock. Values are means ± S.E.M. Asterisks (*) indicate significant difference between treatment groups ($P < 0.05$).

3.4.4 Association between QBA, behavioural and breech soiling parameters

QBA scores were analysed together with breech soiling measures and quantitative behaviour measures through PCA, revealing two main components explaining 50.2% and 14.6% of the variation between sheep, respectively. Figure 3.2 shows the loadings of the 14 variables on these two main components. On PCA component 1, the first GPA dimension (positively occupied; -0.31) and percentage grazing (-0.31), showed the highest negative loadings, whereas urine stain scores (0.34), dag scores (0.33), size of flystruck area (0.33), restlessness (0.32), dag moisture scores (0.31), abnormal (0.29), head turning (0.24), tail wagging (0.25) and biting rump region (0.24) showed the highest positive loadings. These associations are supported by significant correlations between individual QBA dimension 1 scores, indicating that sheep that spent less time grazing (R_s = 0.79; P < 0.001), more time standing abnormally (R_s = -0.76; P < 0.001), displayed higher levels of overall restlessness (R_s = -0.75; P < 0.001), and engaged in kicking (R_s = -0.35; P < 0.05), head turning (R_s = -0.56; P < 0.01), and biting rump region (R_s = -0.61; P < 0.01) were perceived by observers as more *'exhausted/irritated'*. In addition, size of flystruck area (R_s = -0.79; P < 0.001) and dag score (R_s = -0.51; P < 0.01) were negatively correlated with GPA dimension 1 scores. On PCA component 2, kicking (-0.61) and percentage walking (-0.25) showed the highest negative loadings, whereas GPA 2 scores (inquisitive; 0.43), head turning (0.34), tail wagging (0.30), biting rump region (0.28) and GPA 1 scores (positively occupied; 0.19) showed the highest positive loadings. These associations were partially supported by significant correlations between individual QBA dimension 2 scores, where sheep that spent more time walking (R_s = 0.43; P < 0.05), and tended to engage in tail wagging (R_s = 0.50; P < 0.05), head turning (R_s = 0.38; P < 0.05), biting rump region (R_s = 0.39; P < 0.05), and kicking (R_s = -0.34; P < 0.05), were perceived by observers as more *'inquisitive/collected'*.

As done during the GPA process, the positions of the assessment animals were plotted to summarise the assessment of these animals on the basis of all recorded variables (Figure 3.3). The distribution of these sheep on the two main PCA components were interpreted using the PCA configuration and loadings presented in Figure 3.2, such that the components were characterised by those variables with the highest and lowest loadings. This plot shows a clear grouping of sheep by treatment on PCA component 1 (Figure 3.3).

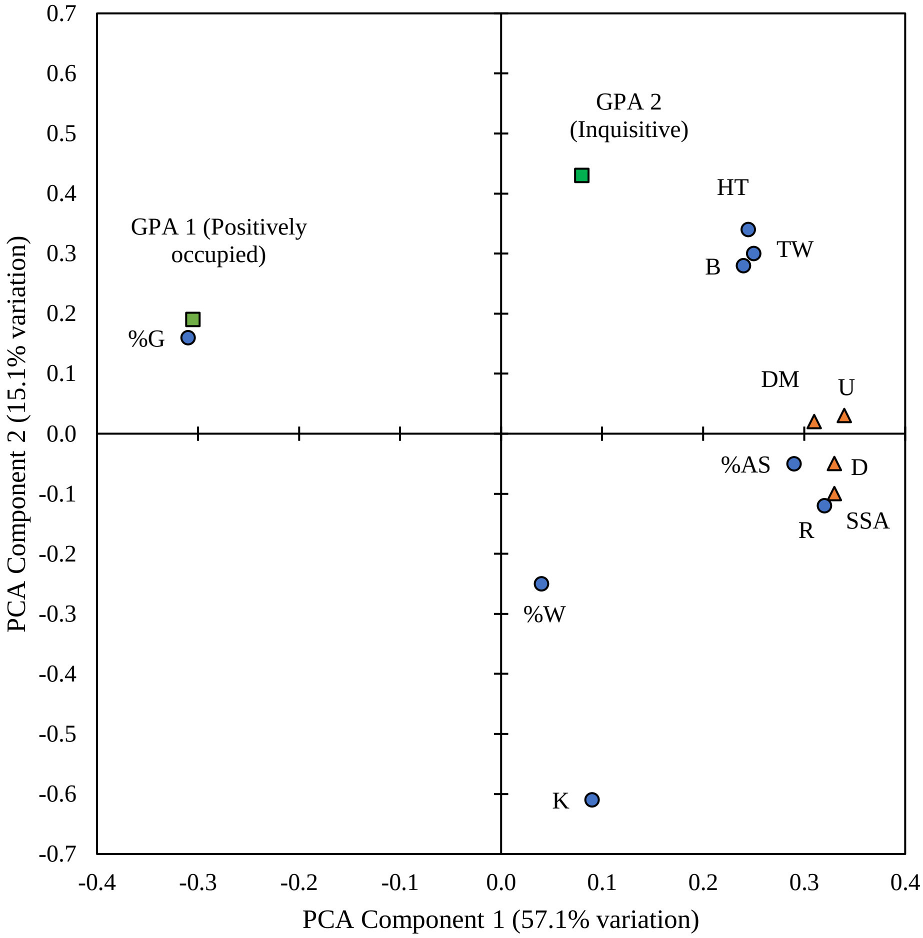

Figure 3.2. Summary of Principal Component Analysis (PCA) dimensions comparing scores for breech soiling variables, quantitative behavioural scoring, and Qualitative Behavioural Assessment (QBA). Breech soiling variables (orange triangles): Dag score (D), Dag moisture score (DM), Urine stain score (US), and Size of flystruck area (SSA). Quantitative behaviour scoring (blue circles): Abnormal standing (AS), Grazing (G), Biting rump region (B), Head turn (HT), Kicking (K), Tail wagging (TW), Restlessness (R), and Walking (W). QBA (green squares): Generalised Procrustes Analysis (GPA) scores on dimensions 1 and 2.

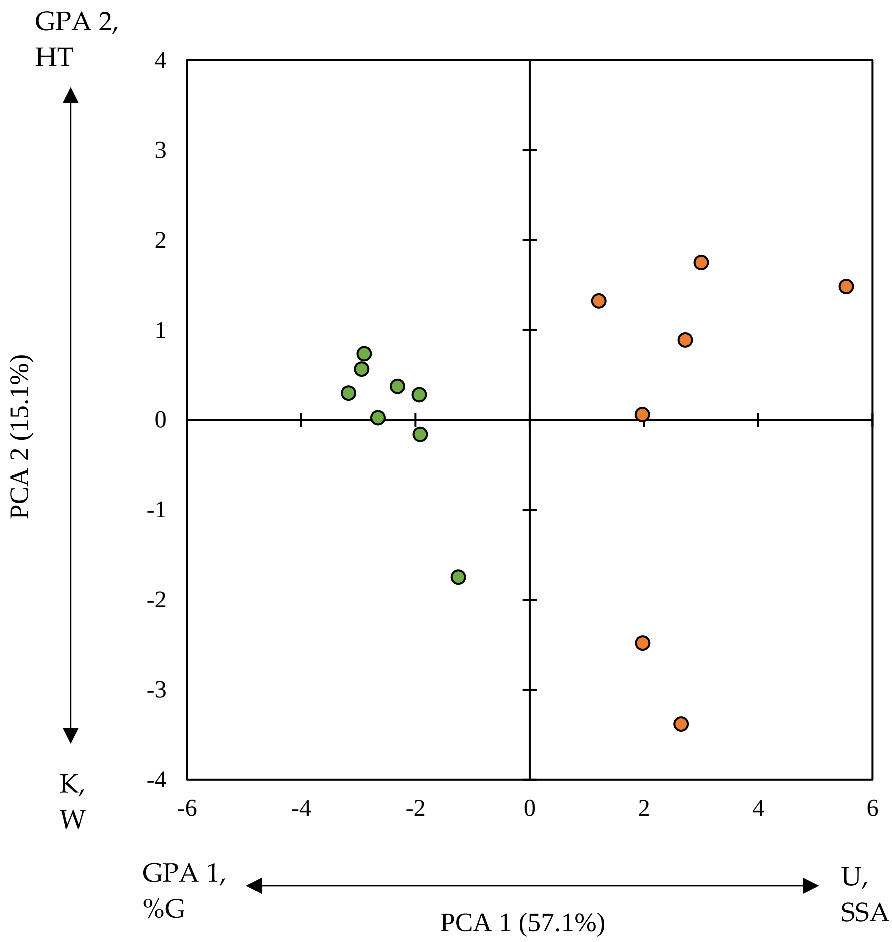

Figure 3.3. Position of individual sheep from the Flystruck (orange marker) and Non-flystruck (green marker) groups on the two main Principal Component Analysis (PCA) dimensions characterised by Urine stain score (U), Size of flystruck area (SSA), GPA 1 (Positively occupied), Grazing (%G), Kicking (K), Walking (%W), GPA 2 (Inquisitive) and Head turning (HT). Complete loadings for PCA dimensions 1 and 2 are summarised in Figure 3.2.

3.5 DISCUSSION

The diagnosis of flystrike is usually straightforward based on physical examination of individual sheep, but there is an obvious need for non-intrusive method of identification that does not involve the gathering and individual handling of all animals. Such a method would allow for the targeted or selective treatment of animals, reducing costs and minimising labour as it would involve only the gathering, handling and treatment of flystruck animals rather than all stock. Here, we applied both quantitative and qualitative methods to study the behaviour of breech-flystruck sheep in a paddock setting to identify potential indicators of flystrike. The significant finding of this study was that flystruck sheep displayed observably different behaviour compared with non-flystruck sheep. First, flystruck sheep displayed different behaviour profiles, characterised by more restless behaviour, the adoption of abnormal postures and reduced grazing, indicating that flystrike causes distress. Second, flystruck animals displayed an *'exhausted/irritated'* demeanour as assessed by observers who were blinded to the experimental treatment. Third, the behaviour scoring and demeanour of animals were associated with physical flystrike parameters (breech soiling variable; size of flystruck region and dag score), highlighting the biological relevance of these observations. Together, these findings suggest that non-intrusive monitoring of specific behaviours in a formal manner may prove a useful tool for producers to identify breech-flystruck sheep.

3.5.1 General quantitative behavioural responses of sheep to breech flystrike

Only flystruck sheep bit or attempted to bite their rump region in the present study, suggesting that such behaviour is of pathological origin. The prolonged or intense attention to an area in the form of biting, licking or rubbing can be an indicator of pain and distress in animals (National Research Council, 2009). Such behaviour may be an attempt to alleviate painful, itching and/or irritating sensations, or perhaps represents an attempt by the sheep to remove the source of the noxious stimulus caused by the feeding activity of the maggots, accumulative damage to the skin and underlying tissues, and ensuing inflammatory response. These biting behaviours were distinctive and only occurred in flystruck sheep, suggesting that they could prove to be useful indicators of breech flystrike.

Flystruck animals also displayed more 'restless' behaviour than non-flystruck sheep, having a near 4-fold increase in the expression of abnormal behaviours that interrupted their predominant behaviour (walking/grazing/standing). The combination of kicking, head shake, head turn, biting rump region, and tail wagging provided a good index for the identification of sheep with flystrike in the present study. These results are consistent with studies of behavioural responses of lambs to painful husbandry procedures (Thornton and Waterman-Pearson, 1999; Molony et al., 2002; Grant, 2004; Lomax et al., 2010), where combined or integrated scores were useful in distinguishing pain responses in lambs. It is thought that the cumulative scoring of active behaviours into a single index, such as the present restlessness score herein, may compensate for differences in the expression of the pain response between individuals subject to the same challenge (Molony et al., 2002). Moreover, such scores may also compensate to some extent, the fact that animals can display such behaviours as part of their normal repertoire. This was seen in the present study with both flystruck and non-flystruck animals observed to kick, wag their tails and shake their heads, although fewer non-struck animals tended to display such behaviour. Perhaps a combined behaviour index that reflects the intensity of expression would prove more useful for the identification of flystrike than simply quantifying separate behaviours. This may also allow for easier discrimination between animals and compensate, at least in part, for the occurrence of behaviours in unchallenged animals.

Flystruck sheep spent less time grazing than non-flystruck sheep and tended to adopt abnormal postures while standing. Reduction in grazing time was a consequence of the repeated expression of abnormal, presumably pain-related, behaviours, as evidenced by extreme 'restlessness' observed in the flystruck animals. Broadmeadow et al. (1984) report that flystrike is accompanied by high fever, resulting in reduced feed intake in sheep. Fever and inappetence could contribute to the observation of reduced grazing in our study. From a welfare perspective, reduction in grazing, a behaviour that animals would be considered highly motivated to perform, is both important and concerning. Flystruck sheep also spent a larger proportion of time standing abnormally, which is consistent with the adoption of abnormal postures when standing in lambs in response to castration and mulesing (Fell and Shutt, 1989; Molony et al., 2002; Grant, 2004), indicating discomfort and/or pain. While observations of grazing and abnormal postures may provide useful information in terms of welfare, they are neither unique to breech flystrike occurring in a broad range of situations nor feasible due to the amount of time such observation would require. Thus, for such measures to prove useful in the identification of flystruck animals for treatment they would need to be incorporated into a larger assessment scheme and be remotely captured.

Not all of the behavioural parameters recorded in the present study proved useful. Indeed, several of the proposed pain-related behaviours (kicking/head turn) were observed infrequently in the flystruck group (less than 50% of animals) or were also observed in the unchallenged, non-flystruck group. It is important to consider that most of the behavioural indicators of pain in sheep were developed to assess lambs undergoing common painful husbandry procedures (e.g. castration, tail docking and mulesing). As such, they may not adequately reflect pain responses in adult animals or the type of injury and presumed pain caused by the disease.

3.5.2 Qualitative behavioural assessment

Differences in behavioural expression were observed between two groups of sheep, where observers scored the flystruck sheep as more *'exhausted/irritated'* compared to non-flystruck animals (which were scored as more *'positively occupied/assured'*). Changes caused by immunological responses designed to counter infestation and associated toxaemic challenge, fever, in particular, are often energetically costly (Colditz, 2002; Broom, 2006), and affected animals may consequently appear lethargic or fatigued and uninterested in their surroundings. Sick animals are frequently described as lethargic or depressed and this lethargy is generally associated with the fever response (Hart, 1988). It is likely that the 'irritated' demeanour in flystruck animals reflects the negative emotional state associated with the presence of painful cutaneous lesions or the active feeding of the maggots causing irritation, pain or discomfort. Disease and injury are generally considered to have emotional components and studies have suggested that QBA scores can reflect the deleterious effect that injury or disease has on emotional state in pigs (Camerlink et al., 2016), cattle (de Boyer des Roches et al., 2018) and sheep (Phythian et al., 2016). While we cannot directly observe or assess psychological welfare, it has been suggested that QBA offers insight into emotional (psychological) state by summarising how animals perceive and interact with their environment through assessments of body language or behavioural expression (Boissy et al., 2007; Rutherford et al., 2012). The relationship between emotional state, behavioural expression and disease may not be a simple one, but it is clear from these results that breech flystrike had a negative effect on the animals that led to altered behavioural expression as identified by observers using the QBA methodology.

3.5.3 Associations between breech soiling assessments, behavioural scoring, and QBA

Quantitative behavioural scores and scores for behavioural expression (QBA) converged towards the same interpretation, indicating that flystruck sheep are more distressed compared with non-flystruck animals. The behavioural response of sheep suffering breech flystrike is characterised by an *'exhausted/irritated'* demeanour, comprising of the expression of a combination of different active behaviours with abrupt movement of the head, tail or limbs (head turning, biting, tail wagging and restlessness), the adoption of abnormal postures while standing and reductions in grazing behaviours. Furthermore, analysis indicates that these responses are related to the irritation or pain caused by tissue damage (strike severity scores) and degree of breech soiling in the animal (dag score and urine score), highlighting the biological relevance of these assessments. How individual sheep expressed pain or irritation caused by flystrike in the present study appeared to be variable, consequently the complex behavioural profile of animals suffering breech flystrike may have been difficult of capture or interpret using purely quantitative methods. Such variability has also seen in the pain responses of lambs to rubber ring castration and tail docking (Molony et al., 2002). That observers were able to successfully differentiate animals using QBA, and that those scores combined meaningfully with quantitative data in the present study, adds value to QBA methodology as a holistic, 'whole-animal' approach. Thus, QBA descriptors are suited to pick up responses to breech flystrike that may go undetected when behaviour is quantified in an isolated manner (e.g. quantification of kicking or head turning events). It appears that a combined score of those behaviours indicative of restlessness and assessment of demeanour using QBA provided the best individual discriminatory basis between animals and may be reliable indicators for the practical identification of breech flystrike in sheep.

3.5.4 Considerations for practical application

From a practical viewpoint, the development of a small number of indices that indicate flystrike remotely would be ideal for sheep producers. Although preliminary in nature, the results herein suggest that while the behavioural presentation of flystrike varied between animals, the common denominator amongst the affected animals was the presence of agitation or restlessness, as evidenced by the intense expression of restless behaviours and the *'exhausted/irritated'* demeanour. It appears that the altered behavioural expression associated with breech flystrike is obvious and easily identifiable from video footage as short as 20 s duration. Indeed, whether in person or from remote video footage, observations required for QBA are considered quicker than traditional behavioural recording (Knierim and Winckler, 2009; Phythian et al., 2016; Hintze et al., 2017; Grant et al., 2018; Minero et al., 2018). Moreover, QBA has the potential to act as an initial screening tool (Fleming et al., 2016), thus when used during routine monitoring may provide producers with an early warning of flystrike and would allow producers to identify those animals in need of closer evaluation and if required, treatment. Overall, it is likely that stock persons informally use these behaviours to inform choices to visually inspect stock for flystrike, and the work presented herein validates these observations. Perhaps the incorporation of formal scoring and training to improve recognition of these behaviours would advance observational skills and facilitate the more prompt and accurate identification of animals in need of treatment on-farm.

The use of the comprehensive assessment of body language provided by QBA coupled with the more specific information offered from behavioural indices of restlessness and grazing are well suited to automated capture. There have been remarkable advancements and success in the development of biosensors to detect behavioural changes across various species (Rutten et al., 2013; Matthews et al., 2016; Fogarty et al., 2018). It is possible that such technologies could be adapted to detect those behaviours presented here. For example, Williams et al. (2018) reported preliminarily data that suggests accelerometers attached by way of neck collars could identify drinking behaviour in cattle though the detection of head-neck position changes. Perhaps this or a similar technology could be modified to allow for the identification of postural change of the neck indicative of orientation of head biting the rump region, and/or to quantify grazing behaviour.

3.5.5 Limitations and future work

Several additional steps need to be taken in order to assess the potential relationship between behavioural expression and breech-strike and to further conclude on the diagnostic value of behavioural indices for practical identification of flystruck sheep for treatment. For example, as the quantitative behavioural observation in the present study was based on short observational periods, a more detailed observation of the behaviour in a larger sample of flystruck animals over a longer period of time is needed to classify behaviours as definitively indicative of breech-flystrike. Furthermore, a study investigating changes in the behaviour, behavioural expression and production performance of sheep suffering strike of varying severity (degrees of damage to tissues) would be valuable to identify behaviours for early detection. Likewise, the inclusion of physiological parameters is vital for the understanding and support of the findings in the present study. For example, assessments of the activity of the sympathetic nervous system such as heart rate and respiration, in addition to traditional measures of the hypothalamic-pituitary-adrenal system could be valuable as they are considered indicators of pain and distress, and have been applied across a variety of animal models (reviewed by Landa, 2012; Gigliuto et al., 2014). It is also crucial to consider that there are different types of flystrike that need to be monitored on-farm and these types of flystrike may impact the behaviour of sheep or present differently. Since accurate monitoring of stock for all types of flystrike is necessary on-farm, this is an area where future work is required. Nonetheless, the data presented here provides proof of concept that assessments of behaviour provide useful information that could be used to identify breech-struck sheep on-farm.

3.6 CONCLUSIONS

In the present study, flystruck animals spent less time grazing, had abnormal posture when standing, exhibited head turning and biting of the rump region, displayed higher levels of restlessness, and were perceived to be more *'exhausted and irritated'* compared to non-flystruck sheep. These behaviours are likely to be pain-related and these results, in agreement with general considerations, suggest that flystrike is painful and causes clear distress in sheep. More extensive studies are still required to further validate the relationship between flystrike and these pain-related behaviours, and to refine the use of these behaviours to create an index for the identification of flystrike. However, the results of this study suggest that the behaviour of breech-flystruck sheep diverges markedly from normal patterns and these differences are easily observable. Thus, the formal monitoring of animals for these behaviours represents a potential tool for the initial remote identification of sheep suffering breech-strike.

CHAPTER 4 BEHAVIOURAL ASSESSMENT OF SHEEP IS SENSITIVE TO LEVEL OF GASTROINTESTINAL PARASITE INFECTION

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4.1 ABSTRACT

Qualitative behavioural assessment (QBA) was applied to investigate the expressive behaviour of sheep with varying intestinal parasite burdens over two experiments. The expressive behaviour of sheep naturally infected with intestinal parasites was assessed from video footage collected in the paddock, and assessments were compared pre- and post-treatment with anthelmintic drench. The first experiment assessed sheep with a range of parasite burdens ($n = 28$), and the second compared sheep that expressed clinical symptoms of parasitism (Anaemic, $n = 5$) with those that did not (Non-anaemic, $n = 5$). Behavioural expression scores were validated against individual clinical evaluations (faecal egg counts – FEC, faecal consistency and anaemia scores), production parameters (body mass and body condition score), and quantitative locomotive measures (walking speed and return order to paddock). Twenty-two observers scored 28 video clips using QBA in experiment 1, and in the second experiment, 35 observers scored 20 video clips that depicted the 10 focal sheep pre- and post-treatment. QBA scores were analysed using Generalised Procrustes Analysis (GPA), and sheep scores on the main GPA dimensions were evaluated in relation to parasite burden using Spearman Rank correlations and repeated-measures ANOVA for experiment 1 and 2, respectively. In both experiments, observers reached significant ($P < 0.001$) consensus in their assessment of the behavioural expression of sheep. In experiment 1, sheep with higher FEC had lower anaemia scores (indicative of anaemia), poor body condition, lower body mass, walked slower, ranked lower in return order, and were scored by observers as more *'docile/at ease'*, and more *'assertive/motivated'* on GPA dimensions 1 and 3, respectively ($P < 0.05$). In experiment 2, observers scored Anaemic sheep as significantly more *'unsettled/apprehensive'* compared to Non-anaemic sheep on GPA dimension 1 ($P < 0.05$). In addition, observers consistently scored Non-anaemic sheep as more *'bright/observant'* following treatment on dimension 2 ($P < 0.001$). On dimension 3, all animals were scored by observers as less *'depressed/suspicious'* post-treatment ($P < 0.05$). An increase in average walking speed across all sheep was also identified post-treatment ($P < 0.05$). We conclude that under the conditions tested, QBA could provide information on the behavioural expression of sheep related to varying parasite burdens and the impact of treatment. The data presented herein offers proof of concept that assessments of behavioural

expression could aid producers in the monitoring and management of parasitised sheep on-farm in terms of identification of individuals for treatment, and efficacy of anthelmintic treatment.

4.2 INTRODUCTION

Gastrointestinal parasitism is one of the most common and important infections in sheep (Lane et al., 2015). Sheep infected with intestinal parasites can have reduced wool growth, milk production and weight gain, and subclinical cases are also associated with reduced performance due to reductions in voluntary feed intake and efficiency of feed utilisation (Coop and Holmes, 1996; Mavrot et al., 2015). Aside from the production implications, intestinal parasites are also a health and welfare concern as they cause scouring (diarrhoea), anaemia, loss of body condition, and in severe or untreated cases, death (Miller et al., 2012).

Typically, the management of intestinal parasites involves the anthelmintic treatment of all animals within the flock at strategic intervals on the basis of regular faecal egg counts (FEC) (Miller et al., 2012). However, this management strategy has resulted in the development of anthelmintic resistance in sheep worldwide (Stafford et al., 2009). Selective or targeted treatment of animals has become the 'ultimate goal' with the strategy aiming to minimise the impact of parasites and also reduce the rate of development of anthelmintic resistance (Besier and Love, 2003; Lane et al., 2015). Targeted selective treatment relies on the identification of animals that are likely to benefit from treatment, leaving the animals able to cope with parasites untreated (Kenyon et al., 2009; Kenyon and Jackson, 2012). To date, there have been a number of markers investigated as indicators for worm treatment with varying degrees of success, including FEC, Faffa Malan Chart (FAMACHA©) anaemia score, body condition score changes, body-mass changes, and diarrhoea or dag score (as reviewed by Kenyon and Jackson, 2012). With the exception of FEC, the FAMACHA system has been the only practical proven on-farm method to identify animals for treatment (Bath and van Wyk, 2009). However, these methods for confirmation of parasite infection can be both disruptive for the sheep and time consuming for the staff. The time delay between sample collection and diagnosis also makes it difficult to return to the individual sheep that require treatment. As such, the sheep industry is interested in developing alternative methods for detection of sheep with high intestinal parasite burdens. While the investigation and development of markers has been a priority, few studies have investigated the application of behavioural assessments for this purpose.

Behavioural assessments can be used in the early detection or pre-clinical diagnosis of health issues (Rutherford, 2002; Dawkins, 2004; Gougoulis et al., 2010). For example, time spent feeding or grazing can be used as an indicator of gastrointestinal abnormalities (Gougoulis et al., 2010). Similarly, the isolation of an individual from its group is often an indication of illness (Constable et al., 2017). With regard to intestinal parasites, it is the generally accepted dogma that most animals will not show any obvious signs of disease prior to the manifestation of clinical symptoms (Miller et al., 2012). This poses a problem since it is vital from a welfare standpoint to identify individuals with subclinical infections for treatment to prevent the progression into clinical cases. As such, for behavioural assessments to help improve the success of parasite management programs, they will need to provide a timely and accurate diagnosis of individuals with a subclinical parasite burden.

Qualitative Behavioural Assessment (QBA) is a 'whole animal' approach that characterises animals through their expressive behaviour, or body language (Wemelsfelder et al., 2000; Wemelsfelder and Lawrence, 2001). In doing so, QBA integrates and summarises details of behaviour, posture and movement, and the context in which these behaviours occur (Wemelsfelder et al., 2001). The integrative and dynamic nature of this approach allows QBA to identify subtle differences in behavioural expression between animals (Fleming et al., 2016). For example, sheep naïve to transport were described by observers as more *'alert/anxious/aware'* and had corresponding increases in heart rate, heart rate variability, core body temperature and stress leukogram, than habituated animals (Wickham et al., 2012). Likewise, Grant et al. (2018) reported that when traversing a walk-over-weigh setup, sheep that were described by observers as more *'focused/collected/assured'* displayed fewer abnormal or escape behaviours (e.g. baulking and circling events), had normal head posture and took less time to complete the given task. Furthermore, the utility of QBA has been supported by many studies in various livestock species demonstrating significant associations with standard behavioural and physiological measurements relevant to sheep welfare assessment (reviewed by Fleming et al., 2016).

Since the success of targeted treatment for parasite burdens is dependent on the sensitivity and accuracy of indicators to identify sick animals, intestinal parasites represent an important model to test the sensitivity of the QBA methodology as a measure of the behavioural expression of sheep. Here we report the results of two experiments intended to investigate the expressive behaviour of sheep with varying intestinal parasite burdens and the impact of anthelmintic treatment. The first experiment investigated whether the behavioural expression of sheep naturally infected with gastrointestinal parasites differed with infection level. The second experiment evaluated the behavioural expression of sheep that expressed other clinical symptoms of parasitism caused by high FEC and those that did not, and compared assessments pre- and post-treatment with anthelmintic drench.

4.3 MATERIALS AND METHODS

These experiments were approved by the Animal and Human Ethics Committees at Murdoch University (R2598/13; O2780/15; 2008/021) to ensure compliance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, the Australian Code for the Responsible Conduct of Research 2007, and the National Statement on Ethical Conduct in Human Research, 2007. All experiments were conducted at Murdoch University Research Farm, located in Murdoch, Western Australia (WA) (Latitude: -32.03º S; Longitude: 115.50º E).

4.3.1 Animals and general handling procedures

The two experiments examining the effect of gastrointestinal parasitism on sheep behaviour were carried out over 23 days in the late autumn period (April and May). In both experiments, sheep were selected from a mixed Merino and Merino-cross group of 53 mixed-sex individuals naturally infected with gastrointestinal parasites. Focal animals were maintained and managed within the source flock throughout the study and could be visually identified from conspecifics by side brands painted on the day of selection (Day 0), and pre-existing radio-frequency identification (RFID) tags. All 53 sheep were subject to clinical evaluations and treatment during the study, and those focal animals in each experiment were subject to additional behavioural assessments. Following routine farm practice, all animals (experimental or otherwise), were treated for intestinal parasites using the broad-spectrum anthelmintic (Cydectin©, moxidectin 1 mg/mL, Virbac Australia) with, as per standard protocol, a dose rate of 0.2 mg moxidectin/kg body weight, based on the heaviest sheep in the mob, given on Day 7. Aside from clinical evaluations (Days 0 and 21), treatment (Day 7) and collection of footage for behavioural assessment (Days 1 and 22), the focal animals for each experiment were free to roam within an enclosed 2.6 ha paddock. Animals had ad libitum access to clean water and pasture, with supplementary feeding as required.

4.3.1.1 Clinical evaluation

On Days 0 and 21, each animal (experimental or otherwise) was subject to a clinical evaluation to determine the level of parasite burden and evaluate intestinal health. An experienced veterinarian was responsible for the clinical evaluations and collection of faecal samples. Faecal samples were collected from animals manually restrained, with approximately two grams of fresh stool collected directly from the rectum of each animal. Samples were immediately stored on ice and were later refrigerated. Within 4 days following collection, the samples were examined to provide an estimate of parasite egg numbers and identity using FEC. Egg counts were carried out on each sample using the modified McMaster technique described in the Australian Standard Diagnostic Techniques for Animal Disease Manual (Corner et al., 1993), with a sensitivity of 50 eggs per gram (epg). In the present study, eggs were classified into the following categories by two experienced technicians; *Eimeria* (coccidia) spp. *Strongyloides* spp., *Trichuris ovis*, *Moniezia expansa*, Strongylids, *Nematodirus* spp., and other. Strongyle-type oocytes and *Nematodirus* spp. eggs were counted and reported separately. All subjects selected for this study were found to be naturally infected with Strongylid-type nematodes and *Eimeria* spp., however, for the purposes of this study the presence of *Eimeria* spp. was noted but not enumerated and their presence did not contribute to the reported Total FEC. The contribution of Strongylid eggs to Total FEC averaged 86.7 % across the study.

Faecal consistency scores (FCS) were taken from individual faecal samples to assess intestinal health and indicate the presence of faecal soiling, or diarrhoea. The consistency of faecal samples were scored using a five-point scoring method (Barrett et al., 1998), where 1 indicated hard faecal pellets and 5 indicated watery fluid faeces (diarrhoea). As faecal consistency is known to influence egg counts, the FEC reported in the present study were corrected using FCS as per Le Jambre et al. (2007).

Anaemia scores based on the colour of the ocular conjunctivae in sheep can be used to identify those individuals that cannot cope with worm infection (Malan et al., 2001). In the present study, a modified three-point FAMACHA© scoring system was used to assess the colour of the ocular membranes. Sheep were classified into one of the following categories; white (1), pink (2) and red (3).

4.3.1.2 Production parameters

Prior to release following clinical evaluations, measures of body mass and body condition score (BCS) were taken. Individual body weights were obtained for all animals using an electronic scale. BCS was measured by spinal palpation of the lumbar and sternal region using the 1 – 5 scale (1 = emaciated, 5 = fat) as described by Russel (1984). To maintain consistency, measurements were always performed by the same assessor.

4.3.1.3 Locomotive parameters

For locomotive parameters, video footage was collected from the animals for analysis. Assessments of locomotive activity, specifically walking speed (m/s) and return order, were made on each animal as they returned to their paddock, under their own volition, after clinical evaluations were held. A single video camera (Panasonic HC-W570M: Panasonic Corporation, Kadoma, Japan) was placed to capture continuous footage as the sheep returned to the paddock. The video footage collected was used to ascertain:

- The order in the mob of each of the 10 assessment sheep as they moved under their own volition; and
- The average speed (m/s) at which the sheep walked (measured over a distance of approx. 70 m).

4.3.2 Experiment 1

The purpose of this experiment was to investigate how the behavioural expression of sheep naturally infected with gastrointestinal parasites as measured by QBA differed with infection level. The subjects in this experiment were 28 adult Merino sheep of mixed-sex (14 wethers and 14 ewes) selected from the source flock. On Day 0, each animal was subject to an initial clinical evaluation to determine the level of parasite burden and evaluate intestinal health. Video footage was collected from these 28 sheep both returning to paddock (Day 0) and the following day (Day 1) for locomotive and QBA analysis, respectively.

4.3.3 Experiment 2

The purpose of this experiment was to investigate whether the behavioural expression of sheep displaying clinical symptoms of gastrointestinal parasitism differed from those animals with levels of infection that did not result in the expression of clinical symptoms and whether these assessments would differ following anthelmintic treatment. Ten ewes were selected for this experiment, with five individuals identified displaying clinical symptoms of infection (i.e. anaemia and/or diarrhoea) as evidenced upon clinical evaluation on Day 0 of the study (Table 4.1). Due to high faecal parasite load estimates and lack of evidence to indicate diarrhoea in the focal animals (Table 4.1), mucous membrane anaemia scores were used to classify animals in the present experiment. Consequently, the focal animals were assigned to two experimental groups; Anaemic (n = 5) and Non-anaemic (n = 5), based on their initial estimated parasite burden and anaemia scores (Table 4.1). Briefly, Anaemic sheep were judged by the veterinarian as having pink or white conjunctivae (i.e. anaemia score < 3) and had an estimated parasite load that ranged from 3,268 to 36,058 epg (mean = 15,030), whereas sheep allocated to the Non-anaemic group did not exhibit anaemia or poor faecal consistency, and had an estimated FEC that ranged from 700 to 5,202 epg (mean = 2,437).

Following the protocol above, the 10 assessment sheep were subject to an additional clinical evaluation on Day 21, and subsequent video collection for QBA assessment on Day 22. Prior to footage collection, the visual identification brands painted on the side of focal animals were altered to maintain observer blindness during behavioural assessments. To maintain consistency, the same assessor performed measurements throughout the study with the exception of the observers used in QBA.

Table 4.1. Overview of clinical evaluation and quantitative assessment parameters of focal Merino sheep in experiments 1 and 2.

Parameters	Experiment 1 (*n* = 28)		Experiment 2 (*n* = 10)			
	Value	Correlation (R_s) with FEC	Non-anaemic		Anaemic	
			Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Clinical evaluation						
Total FEC ǂ (epg)	6,493 (< 50 – 19,300)		2,437 ^{aA} (700 – 5,202)	< 50 ^{aB}	15,030 ^{bA} (3,268 – 36,057)	288 ^{aB} (< 50 – 975)
Faecal consistency (1 – 5)	1.3 ± 0.5	0.23	1.2 ± 0.4 ^{aA}	1.8 ± 0.8 ^{aA}	1.4 ± 0.5 ^{aA}	1.4 ± 0.5 ^{aA}
Anaemia (1 – 3)	2.9 ± 0.4	**-0.39****	3.0 ± 0.0 ^{aA}	3.0 ± 0.0 ^{aA}	1.8 ± 0.4 ^{bA}	3.0 ± 0.0 ^{aB}
Production parameters						
Body condition (1 – 5)	1.9 ± 0.1	**-0.42****	1.9 ± 0.3 ^{aA}	2.2 ± 0.5 ^{aA}	1.8 ± 0.2 ^{aA}	2.0 ± 0.3 ^{aA}
Body mass (kg)	29.7 ± 2.1	**-0.33***	28.7 ± 10.3 ^{aA}	30.5 ± 8.6 ^{aA}	26.9 ± 3.9 ^{aA}	28.4 ± 6.4 ^{aA}
Locomotive parameters						
Walking speed» (m/s)	1.2 ± 0.04	**-0.41****	1.2 ± 0.03 ^{aA}	1.3 ± 0.04 ^{aB}	1.2 ± 0.07 ^{aA}	1.3 ± 0.04 ^{aB}
Order of return	14.5 ± 8.2	**0.41****	3.8 ± 3.1 ^{aA}	5.6 ± 3.0 ^{aA}	7.2 ± 1.9 ^{aA}	5.4 ± 3.4 ^{aA}

ǂ FEC, total faecal egg count eggs per gram of faeces (epg), including; *Strongyloides* spp., *Trichuris ovis*, *Moniezia expansa*, Strongylids, *Nematodirus* ssp., and 'other'. Values for 'Total FEC' are presented as mean (range) adjusted for faecal consistency.
» Walking speed presented as non-transformed values.
* Correlations between FEC and parameters are presented, with significant effects indicated (* $P < 0.05$, ** $P < 0.01$).
ab Different lower-case superscripts indicate statistical difference between groups (Non-anaemic and Anaemic) within a time point (before or after treatment) ($P < 0.05$).
AB Different upper-case subscripts indicate statistical difference within group (Non-anaemic or Anaemic) between time points (before and after treatment) ($P < 0.05$).

4.3.4 Qualitative behavioural assessment (QBA)

Video footage for QBA was collected in the home paddock on Days 1 and 22, so that sheep had time to recover from the measurement processes to control for potential differences caused by handling and faecal sampling (Hargreaves and Hutson, 1990). The digital camera was positioned to capture footage of each animal as it walked through the paddock. Sheep were not isolated, but individual sheep were visually identified by the camera operator and filmed as the mob was calmly moved by a stock person from one side of the paddock to the other. Filming took place between 7 am and 12 pm on these days to control for potential differences in behaviour related to circadian rhythms in sheep (Wyse et al., 2018).

The footage collected on Days 1 and 22 was edited to depict each focal animal as it walked towards and past the camera, with the animal's image typically filling at least 25 – 50 % of the viewing screen. For experiment 1, the 28 focal sheep had a single corresponding clip, whereas the 10 focal animals in experiment 2 had two clips, with footage of each animal captured before and after anthelmintic drench. The resultant clips were approximately 32 s and 30 s duration for experiments 1 and 2, respectively. As footage was collected of the animals from side and front view, evidence of any diarrhoea a common sign of intestinal parasites in sheep would not have been visually evident to observers.

A total of 57 observers were recruited from Murdoch University staff and students (49 female and 8 male) to assess the sheep videos using the QBA methodology. Of these, 22 observers assessed the 28 clips from experiment 1, and the remaining 35 assessed the 20 clips in experiment 2. Observers were given detailed instructions on completing the QBA scoring sessions but were not given any details on the animals or the experimental design. Observers completed a short survey regarding their past experiences with sheep and other domestic livestock species prior to completion of the QBA procedure. To complete the QBA by means of a Free Choice Profiling (FCP) procedure, observers were required to attend two sessions; a term generation session followed by a quantification session.

The first stage of the FCP methodology requires that observers generate a list of their own descriptive terms for later use in the quantitative session. To do this, observers in both experiments were shown a series of video clips (9 and 12 for experiments 1 and 2, respectively), that were chosen to demonstrate a variety of behavioural expressions to allow observers to describe as many aspects of a sheep's expressive repertoire as possible. After watching each clip, observers were given 2 min to write down terms they thought described the animal's behavioural expression. There was no limit imposed on the number of descriptive terms an observer could generate, but terms needed to describe not what the animal was doing (i.e. physical descriptions of the animal such as vocalising, chewing, tail flicking), but *how* the animal behaved (e.g. *'nervous'*, *'relaxed'*). Subsequent editing of the descriptive terms was carried out to remove terms that described actions, and terms that were in the negative form were transformed to the positive for ease of scoring (e.g. *'unhappy'* became *'happy'*). The result being a unique list of descriptive terms for each observer to be used for quantification in the second session. Each descriptive term was attached to a visual analogue scale (minimum to maximum) in an electronic worksheet (Microsoft Excel 2003, North Ryde, NSW, Australia). The list of terms was randomly arranged, although terms with a similar meaning were not listed together.

In the second stage of the FCP methodology, observers were asked to assess the full set of sheep videos from each experiment. To do this, observers viewed and scored the video clips of the focal sheep using their own unique lists of descriptive terms. Observers were instructed to score each animal's expression using the visual analogue scale, where maximum indicated the animal could not show an expression more strongly and minimum reflected the absence of expression; the distance between the minimum-point and their mark on the scale as reflected the intensity of each animal's expression on that term.

4.3.5 Statistical analysis

For QBA, the distance from the start of the visual analogue scale to where the observer had made a mark for each term was calculated (where minimum = 0 and maximum = 100) and these data were analysed by means of Generalised Procrustes Analysis (GPA) (Genstat 2008, VSN International, Hemel Hempsteat, UK; Wemelsfelder et al. (2000). Statistical analysis considered the QBA data from both experiments separately, such that two GPA consensuses were generated and subsequently analysed. For a detailed description of GPA analysis and output interpretation procedures see Wemelsfelder et al. (2000; 2001).

For experiment 1, the association between FEC and the various clinical, production and behaviours parameters collected including the GPA scores of the 28 focal animals (obtained from QBA) were examined using Spearman Rank Order correlation (Genstat 2008, VSN International, UK). There was little variation evident within the parameters collected (e.g. faecal consistency, anaemia, BCS, body mass, walking speed and return order), thus for the purpose of investigating the relationship between behavioural expression and gastrointestinal parasite infection, only correlations between FEC and GPA scores are reported herein.

For experiment 2, faecal egg counts, faecal consistency scores and anaemia scores were compared between Anaemic and Non-anaemic groups using non-parametric analyses including Mann-Whitney *U* (Z_n; two independent samples) and Wilcoxon matched-pairs test (Z_n; paired samples) to compare between groups and across animals pre- and post-treatment, respectively (Statistica 7.1, StatSoft-Inc., North Melbourne, Vic., Australia). BCS and body mass were analysed by Wilcoxon matched-pairs tests. Walking speed (square-root transformation) and return order were analysed by parametric repeated-measures (RM) ANOVAs. For QBA, GPA scores for dimensions 1, 2 and 3 were normally distributed in experiment 2 and were analysed by parametric RM ANOVAs (Genstat 2008, VSN International, UK).

4.4 RESULTS

4.4.1 Experiment 1

4.4.1.1 Clinical evaluation

Faecal egg counts were varied but high across the sheep in experiment 1 (average: 6,493 epg; range: < 50 – 19,300 epg; Table 4.1). FCS were consistently low across these 28 animals (Table 4.1), indicating solid faeces and providing no evidence for diarrhoea or scouring in any assessed animal. Anaemia scores were consistently high across animals (Table 4.1), with the majority of animals judged by the veterinarian to have healthy red conjunctivae.

4.4.1.2 Qualitative behavioural assessment

The 22 observers participating in the QBA component of this experiment generated a total of 100 unique terms to describe the sheep they were shown (average 15 ± 5 terms per observer; range 5 – 24). The GPA consensus profile explained 36% of the variation between observer scores of sheep and this differed significantly from the mean randomised profile ($t99 = 11.9$, $P < 0.001$). The three main dimensions of behavioural expression explained 35%, 16% and 7% of the variation in scores attributed to individual sheep (GPA 1, 2 and 3, respectively).

The terms from all the observers were pooled for experiment 1 and those with the highest and lowest correlations with each of the GPA dimension are shown in Table 4.2. High scores on GPA 1 were associated with the semantic correlation tags of *'irritated'* and *'responsive'*, whereas low scores were associated with terms such as *'docile'* and *'at ease'*. For GPA 2, high scores were associated with terms such as *'pressured'* and *'timid'* and low scores with terms such as *'inquisitive'* and *'purposeful'*. On GPA 3, high scores were associated with terms such as *'assertive'* and *'motivated'*, whereas low scores were associated with terms such as *'lazy'* and *'social'*. The two highest weighted (loaded) terms for each GPA dimension, as shown in Table 4.2, were selected for the purpose of labelling the GPA dimensions and describing the dimensions in relation to FEC (Figure 4.1).

Table 4.2. Terms for all observers in experiments 1 (observer *n* = 22) and 2 (observer *n* = 35), showing the highest correlation with each end of the Generalised Procrustes Analysis (GPA) dimensions 1, 2 and 3 of the respective consensus profiles. Terms shown have a correlation > 0.5 (high loading) and < -0.5 (low loading) for GPA dimensions 1 and 2, and > 0.3 (high loading) and < -0.3 (low loading) for GPA dimension 3. Numbers in parentheses represent the number of observers that generated and subsequently used that word to assess sheep expressive behaviour.

Experiment 1			**Experiment 2**		
GPA dimension	**Low values**	**High values**	**GPA dimension**	**Low values**	**High values**
1 (35%)	Docile (1), at ease (2)	Irritated (1), responsive (1), alarmed (1), intimidated (1), encouraged (1), enthusiastic (1), proud (2), motivated (1), wary (3), vigilant (3), complacent (1), distressed (1)	1 (25%)	Unsettled (1), apprehensive (2), concerned (2), sad (4)	Eager (4), rushed (2), motivated (2), energetic (5)
2 (16%)	Inquisitive (2), purposeful (1)	Pressured (1), timid (2), hurried (1), weak (1)	2 (17%)	Bright (1), observant (1), collected (1)	Rushed (2), skittish (1), reluctant (1)
3 (7%)	Lazy (2), social (1), displeased (1), responsive (1)	Assertive (1), motivated (1), encouraged (1), dopey (1), weak (1), agitated (4), frazzled (1), startled (1), docile (1)	3 (10%)	Depressed (1), suspicious (1), bored (1), disturbed (1)	Careful (1), reluctant (1), upset (1), timid (1), enthusiastic (1), on edge (1)

4.4.1.3 Relationship between FEC and parameters

Total FEC for the naturally parasitised sheep were significantly correlated across a number of the clinical, production and behavioural measures collected (Table 4.1; Figure 4.1). Specifically, sheep with higher FEC tended to have lower anaemia scores (R_s = -0.39, $P < 0.01$) indicative of pale conjunctivae and anaemia, poorer body condition (R_s = -0.42, $P < 0.01$), lower body mass (R_s = -0.33, $P < 0.05$), have slower walking speeds (R_s = -0.41, $P < 0.01$) and rank lower in return order (R_s = 0.41, $P < 0.01$). With regard to QBA, those sheep with higher FEC tended to receive low scores from observers on GPA 1 (*'docile/at ease'* – *'irritated/responsive'*; R_s = -0.28, $P < 0.05$), and high scores on GPA 3 (*'lazy/social'* – *'assertive/motivated'*; R_s = 0.35, $P < 0.05$).

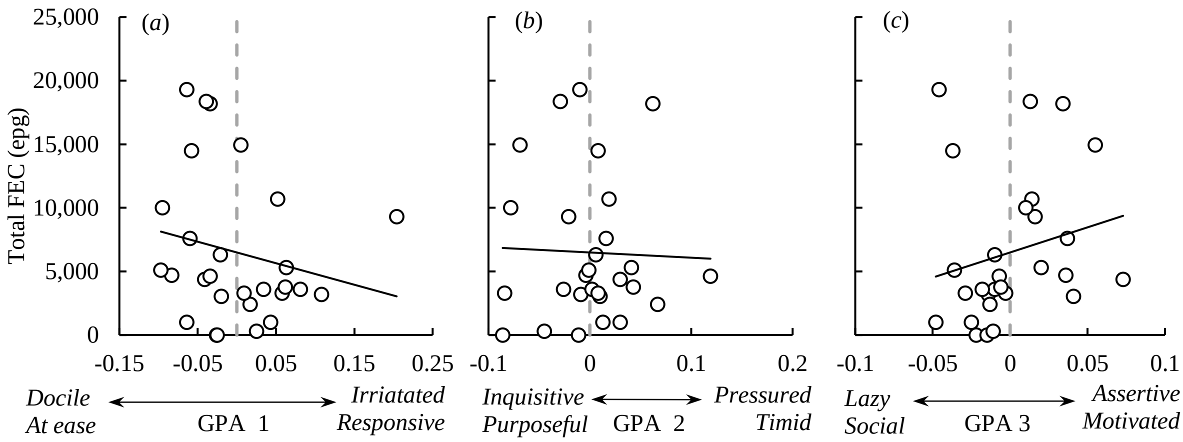

Figure 4.1. Spearman rank correlations between Generalised Procrustes Analysis (GPA) scores on dimensions 1, 2 and 3, and the Total FEC for the 28 focal sheep in experiment 1.

4.4.2 Experiment 2

4.4.2.1 Clinical evaluation

Faecal egg counts of the Anaemic and Non-anaemic sheep pre- and post-treatment are outlined in Table 4.1. Prior to treatment, the estimated level of parasite infection was different between groups ($Z_n = 5 = -2.4$, $P < 0.05$), and anthelmintic treatment appeared effective at reducing parasite burdens across both Anaemia and Non-anaemic sheep ($Z_n = 5 = 2.0$, $P < 0.05$ for both groups; Table 4.1).

The consistency of the faeces did not vary between Anaemic and Non-anaemic sheep pre- or post-treatment ($P > 0.05$; Table 4.1). These scores indicate similarly solid faeces and provide no evidence for diarrhoea or scouring in any assessed animal.

Anaemia scores were significantly different between animals prior to treatment ($Z_n = 5 = 2.9$, $P < 0.01$), with sheep in the Anaemic group reporting lower scores compared to the Non-anaemic group (Table 4.1). The anthelmintic treatment of animals had a significant effect on anaemia scores in the Anaemic animals ($Z_n = 5 = 2.0$, $P < 0.05$), where all sheep were judged by the veterinarian to have healthy red conjunctivae and not suffering anaemia post-treatment (Table 4.1).

4.4.2.2 Production parameters

There were no significant differences in BCS (*P* > 0.05) or body mass (*P* > 0.05) of the sheep in the Anaemic and Non-anaemic groups before or after anthelmintic treatment (Table 4.1).

4.4.2.3 Locomotive parameters – Walking speed and return order

There were no differences observed in the walking speed of sheep as they returned to paddock related to parasite burden (*P* > 0.05; Table 4.1). However, RM ANOVA indicated that there was a significant positive effect of time ($F_{1,8}$ = 17.0, *P* < 0.01) on the walking speed of the sheep between Days 0 and 21 (Table 4.1).

The order in which the sheep moved past the camera in the laneway showed no significant effects of group (*P* > 0.05), time (P > 0.05) or interaction between group and time (*P* > 0.05).

4.4.2.4 Qualitative behavioural assessment

The 35 observers participating in the experiment generated a total of 90 unique terms to describe the sheep they were shown (average 16 ± 4 terms per observer; range 5 – 25). The GPA consensus profile explained 42% of the variation between observer scores of sheep and this differed significantly from the mean randomised profile (t99 = 15.9, *P* < 0.001). Three main dimensions of behavioural expression explained 25%, 17% and 10% of the variation in scores attributed to individual sheep (GPA 1, 2 and 3, respectively).

The terms from all the observers were pooled for experiment 2 and those with the highest and lowest correlations with each of the GPA dimension are shown in Table 4.2. Overall, these word charts were semantically consistent across the 35 observers, with observer terms converging towards similar meanings. High scores on GPA 1 were associated with the semantic correlation tags of *'eager'* and *'rushed'*, whereas low scores were associated with terms such as *'unsettled'* and *'apprehensive'*. For GPA 2, high scores were associated with terms such as *'rushed'* and *'skittish'* and low scores with terms such as *'bright'* and *'observant'*. On GPA 3, high scores were associated with terms such as *'careful'* and *'timid'*, whereas low scores were associated with terms such as *'depressed'* and *'suspicious'*. The two highest weighted (loaded) terms for each GPA dimension, as shown in Table 4.2, were selected for the purpose of labelling the GPA dimensions and describing the dimensions in relation to experimental group; Anaemic and Non-anaemic (Figure 4.2).

Overall, there were significant group effects for the observer scores of the sheep on all three GPA dimensions (Figure 4.2). Sheep that were in the Anaemic group received significantly lower scores on GPA 1, being scored by observers as more *'unsettled'* and *'apprehensive'*, compared to the Non-anaemic sheep (scored as more *'eager'* and *'rushed'*) ($P < 0.05$). There was a significant time effect on the observers' scores on GPA 3 ($P < 0.05$), which were higher for animals in both Anaemic and Non-anaemic groups following anthelmintic treatment compared with before treatment (i.e. all sheep were scored as less *'depressed'* and *'suspicious'* following treatment). In addition, those sheep in the Non-anaemic groups received lower scores on GPA 2 (group by time interaction: $P < 0.001$), being scored as more *'bright'* and *'observant'* by observers following treatment, whilst the observer scores given to those animals in the Anaemic group did not differ on this dimension.

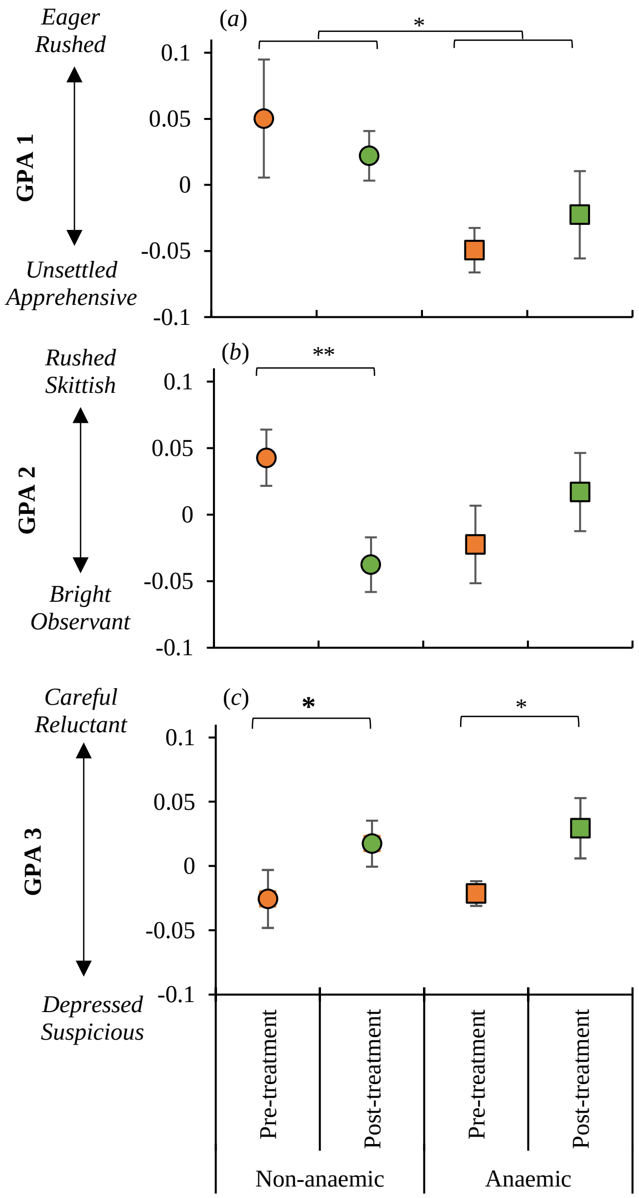

Figure 4.2. Mean (± S.E.M.) of Generalised Procrustes Analysis (GPA) scores on (a) dimension 1, (b) dimension 2, and (c) dimension 3 resulting from assessments of sheep that were in the Anaemic (squares) and Non-anaemic groups (circles), both pre-treatment (orange marker) and post-treatment (green marker) with an anthelmintic drench in experiment 2. Within each dimension, the asterisk identifies significant differences between groups; * $P < 0.05$, ** $P < 0.001$.

4.5 DISCUSSION

The aim of this study was to investigate the application of QBA to assess the behavioural expression of sheep with varying intestinal parasite burdens and the impact of anthelmintic treatment over two experiments. In both experiments, observers reached significant consensus in their assessments of behavioural expression of sheep using the FCP methodology and scores appear to depend on level of parasite infection. In experiment 1, biologically meaningful correlations were found between individual FEC and QBA scores, physical health parameters (anaemia, body condition, body mass) and locomotive parameters (walking speeds and return order). In experiment 2, sheep selected based on the presence of clinical symptoms (Anaemic) exhibited different behavioural expression compared to those without symptoms (Non-anaemic). Furthermore, scores of behavioural expression differed 14 days after treatment, suggesting that assessments are sensitive to improvements in condition caused by reductions in parasite burden, even in sheep with subclinical levels of infection. Although QBA successfully identified subtle differences in the behavioural expression of the focal sheep in experiment 2, the traditional measures of physical health and locomotion, with the exception of walking speed, failed to reveal differences between sheep. This study highlights the potential application of QBA in the practical assessment and monitoring of sheep with gastrointestinal parasite infections.

4.5.1 Experiment 1

The significant finding of experiment 1 was the identification of meaningful correlations between the relative position of sheep on GPA dimensions 1 and 3 and individual FEC. It is generally accepted that parasitised animals will not behave in a similar manner to an unchallenged animal, and any alterations in host behaviour could relate to parasite burden and the impact infection causes within the animal (Poulin, 1995; Szyszka and Kyriazakis, 2013). The tendency of observers to score sheep with higher FEC as more *'docile'* and *'at ease'* on GPA 1 may reflect fatigue or the lower energy state of the sheep. Poorer nutritional status by suppressed voluntary feed intake and/or reductions in the efficiency of nutrient absorption are noted in parasitised animals (Coop and Kyriazakis, 1999; Colditz, 2008). Moreover, the re-direction of resources towards the mounting of an immune response in parasitised animals may influence behaviour (Burgunder et al., 2018). This may translate to lower energy reserves in sheep with high parasite burdens and less active movement. This is consistent with Hutchings et al. (2000), who reported that parasitised sheep are less active than uninfected conspecifics, recording lower rates of movement and reduced grazing times. Likewise, Besier et al. (2016) suggested that as haemonchosis progresses animals will become weaker due to blood loss associated with infection, and therefore may be less likely to move or spend more time laying down. Thus, the scoring of the animals in experiment 1 may reflect a response to conserve energy. For GPA dimension 3, it may be that the tendency for observers to score highly parasitised sheep as more *'assertive'* and *'motivated'* reflects the response to humans present during videoing of behaviour. The presence of humans is stressful to sheep (Romeyer and Bouissou, 1992; Vandenheede et al., 1998), and theoretically, the response of the focal animals may have been influenced by parasite burdens, resulting in the expression of different or more intense behavioural patterns such as flocking or vigilance. Observers may have been interpreted such behaviours as the sheep being attentive to their environment and more 'motivated'. The concurrent stressors in this experiment (human presence and parasite burden) complicates interpretation of behavioural expression, and a more detailed evaluation of these animals' behaviour and physiological responses would be required to verify this interpretation.

Meaningful associations between individual egg counts and various clinical, production and behavioural parameters were also demonstrated in experiment 1. The colour of the ocular conjunctivae and faecal consistency are known to correlate with FEC for some parasite species in sheep (Kaplan et al., 2004; Le Jambre et al., 2007; Seyoum et al., 2018). Indeed, in experiment 1, sheep with paler (pink or white) ocular conjunctivae tended to have higher FEC, although no significant relationship was noted with faecal consistency. While the absence of association between faecal consistency and FEC has been noted elsewhere (Pollott et al., 2004; Mederos et al., 2014), this result may reflect the negligible levels of scouring or diarrhoea in focal animals in the present study. The significant negative correlation of FEC with body condition and weight in this experiment are in agreement with studies that report losses in parasitised sheep not subject to management protocols to suppress worms (e.g. Cornelius et al., 2014). The inverse relationships noted between FEC and the speed and order of in the mob when walking supports informal observations that sheep with heavy parasite burdens suffer from ill-thrift, are slower or more lethargic than their healthier conspecifics and lag behind when 'driven' (e.g. Abbott et al., 2012; Litzow, 2015). It is clear that infection with gastrointestinal parasites has consequences to animals in terms of production and welfare, especially as level of parasitism intensifies.

4.5.2 Experiment 2

Differences in behavioural expression were observed between Anaemic and Non-anaemic animals in experiment 2, where the observers consistently scored the Anaemic sheep as more *'unsettled/apprehensive'* compared to Non-anaemic animals (more *'eager/rushed'*). Parasitic nematode infections are associated with numerous, potentially welfare-compromising symptoms (Miller et al., 2012) and there is evidence for behavioural changes related to gastrointestinal parasitism in ruminants (e.g. Fell et al., 1991; Adams and Fell, 1997; Falzon et al., 2013; Szyszka and Kyriazakis, 2013; Burgunder et al., 2018). Perhaps the differences in the present study relates to the direct impact of infection, or it may be a consequence of the mounted immune response. The difference in expression may reflect lower energy reserves in sheep with clinical parasitism causing less active movement as previously discussed. Alternatively, it may reflect damage to the gastrointestinal tract and the consequential disruption to normal behaviours such as grazing, which may cause outward manifestations of frustration or distress. The inability to perform normal behavioural patterns or motivated behaviours can lead to the development of agitation, restlessness or distress (Wemelsfelder and Farish, 2004). This may make the Anaemic animals appear 'unsettled' to observers. Regardless of the mechanism of altered behavioural expression, these results appear to suggest that the behavioural expression of sheep varies according to its parasite load and that QBA is sufficiently sensitive to detect such changes.

The treatment of sheep with an anthelmintic drench resulted in perceived differences in behavioural expression in both Anaemic and Non-anaemic sheep after 14 days. These differences in perceived behavioural expression on GPA dimension 3 (less *'depressed/suspicious'*) may be attributed to the reduction in parasite burden caused by treatment. Most studies report the improvement of animals following treatment across various parameters including measures of behaviour. For example, Szyszka et al. (2013) reported that the behaviour of cattle subjected to parasite challenge with *O. ostertagi* returned to 'healthy' behaviour patterns within one week of anthelmintic drench. However, in the present case, extrinsic factors such as different weather conditions between assessment points, or the repeated exposure to humans and/or handling leading to habituation, may offer alternative explanations for the apparent 'improvement' in behavioural expression. We note that studies have demonstrated that habituation to humans and transport can influence observer scores of behavioural expression given to sheep (Wickham et al., 2012; Grant et al., 2018). However, due to the limited exposure to humans in the present study, it is plausible that these assessments of behaviour expression are sensitive to improvements in condition caused by reductions in parasite loads. This is supported by observations of increased walking speed in all animals following treatment, indicating that they may have recovered energy reserves, previously directed to management of infection, as their condition improved. Most likely, the interpretation is not so simple and the altered behavioural profile observed after treatment may be due to one or a combination of factors, not easily identified without further investigation.

In experiment 2, the observers perceived an 'improvement' in the demeanour of not only the Anaemic sheep but also the Non-anaemic sheep suffering from subclinical infections after treatment. It is possible that these animals were suffering subclinical infection prior to treatment. The Non-anaemic sheep had established parasite burdens indicating the presences of mature reproducing adult parasites in the gastrointestinal tract, although these animals were without anaemia or poor faecal consistency, and therefore had no visual indicators of infection. The overall effects of subclinical infection are difficult to assess but have been reported to include reductions in performance such as reduced voluntary feed intake and growth rates (Kyriazakis et al., 1994; Kyriazakis et al., 1996), with infection accompanied by potentially costly immune responses (Sykes, 2010). While not as severe as those clinically infected sheep, the condition of animals in the Non-anaemic group may have been compromised. Thus, treatment to reduce parasite burdens may have lessened any stress on the animals, potentially altering how they interacted with their environment and consequently their behavioural expression scores. The 'improvement' in perceived behavioural expression of the Non-anaemic animals following anthelmintic treatment on not one but two dimensions of expression (more *'bright/observant'* on GPA 2, and less *'depressed/suspicious'* on GPA 3), offers support for this. These results suggest that observer scores of behavioural expression in sheep are sensitive to infection level (subclinical and clinical) and the impact of treatment.

Here, the more conventional assessments of body condition and body mass did not reveal a difference between Anaemic and Non-anaemic sheep, nor did anthelmintic treatment result in changes to these parameters. While no difference in body mass of the sheep was found, an average increase in body mass of approximately 13.5% was observed across all the animals 14 days post-treatment, although this just failed to reach significance. Typically, BCS and body mass of parasitised animals are reported to recover following treatment (e.g. Sharma et al., 2016). Though there is evidence that responses are only observed in sheep of low condition (BCS ≤ 2.5) (Cornelius et al., 2014). In the present study, the lack of response to treatment from animals considered to be in low condition (BSC < 2.0) might reflect variability in recovery period due to intensity of pathology or infection type. For example, Angus et al. (1979) reported that two to three weeks was required for the recovery, and presumed normal functioning, of the intestinal tract in sheep infected with *T. colubriformis* larvae. Thus, it is possible that the recovery of infected animals had not progressed enough for improvements to manifest in changes of condition or body mass. Moreover, without knowledge of the infection history (e.g. duration of infection prior to Day 0), it is possible that the pathology experienced by the Anaemic animals had not yet progressed to the point of observable reductions in BCS or body mass, offering an explanation for the lack of differences.

No differences were identified between Anaemic and Non-anaemic animals with regard to walking speed or return order to paddock despite encouraging correlations in experiment 1. Reductions in locomotive activity have been demonstrated in cattle suffering parasite infection (Szyszka et al., 2013). An explanation for the lack of difference in locomotory behaviour between the groups, both before and after treatment, could relate to the absence of the stress of being herded (‘driven’) back to their paddock. In the present study, sheep travelled back to their paddock under their own volition. The significance of this is in the application of these methods for early detection of parasitised animals, which in terms of feasibility for on-farm application would require minimal handling and human involvement. Perhaps the incorporation of accelerometers would prove useful in this regard, having promising results in the ability to identify subtle differences in locomotive behaviour in grazing ruminants (Champion et al., 1997; Barwick et al., 2018b). Indeed, tri-axial accelerometer loggers have recently been used to demonstrate that sheep infected with strongylids display less complex behavioural or activity patterns than uninfected animals (Burgunder et al., 2018). The small sample size in this experiment may be a limiting factor, however, as discussed above, it is also possible that the pathology experienced by the Anaemic animals had not yet progressed to the point of observable reductions in locomotive activity.

While numerous studies have investigated the behavioural expression of sheep, to the authors' knowledge these experiments represent the first investigating gastrointestinal parasite load. The present study expands the application of QBA to the behaviour of sheep with gastrointestinal parasites, as evidenced by the ability of observers to reach consensus and successfully distinguish between sheep with varying levels of parasite burden. Not only does this work bring further evidence that assessments of behavioural expression can be used to assess sheep welfare, but it also highlights the ability of QBA to identify subtle behaviours undetected by traditional behavioural (locomotive measures) and physical measures (BCS and body mass) as suggested in the literature (e.g. Wemelsfelder, 1997; Wemelsfelder et al., 2001; Minero et al., 2016). The data presented here provides proof of concept that assessments of behavioural expression could provide useful information related to parasitism in sheep. However, the authors caution that the ability of QBA to detect significant differences in behavioural expression between treatment groups as reported herein is not the same as being able to use QBA to ascertain the parasitised status of sheep of unknown status. Taken as a whole though, these results suggest that field observations for the monitoring or identification of animals for treatment may be strengthened by the addition of QBA.

4.6 CONCLUSIONS

The assessments of behavioural expression from observers using the QBA methodology in the present study revealed consistent behavioural effects of gastrointestinal parasite challenge. It appears that in using descriptive terminology to summarise details of behaviour, posture and movement, along with the context in which they occur, observers can use QBA to identify changes in behaviour related to both the level of parasite infection and the treatment of infection. These findings indicate that assessments of behavioural expression in sheep may provide useful information in identifying sheep with high parasite burden that require anthelmintic treatment, and therefore opening up the possibility of selective treatment of individuals in a flock. Hence, QBA methodologies have the potential to contribute to reducing the rate of development of anthelmintic resistance.

CHAPTER 5 PRELIMINARY FINDINGS ON A NOVEL BEHAVIOURAL APPROACH FOR THE ASSESSMENT OF PAIN AND ANALGESIA IN LAMBS SUBJECT TO ROUTINE HUSBANDRY PROCEDURES

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5.1 ABSTRACT

The identification and assessment of pain in sheep under field conditions are important, but, due to their stoic nature, are fraught with many challenges. In Australia, various husbandry procedures that are documented to cause pain are routinely performed at lamb marking, including ear tagging, castration, mulesing, and tail docking. This study evaluated the validity of a novel methodology to assess pain in lambs: qualitative behavioural assessment (QBA) was used to compare the behavioural expression of control lambs (CONTROL) with that of lambs subject to these procedures that received either a saline placebo 15 min before procedures (PLACEBO), or were administered meloxicam 15 min before procedures in addition to the standard analgesic Tri-Solfen at the time of procedures, as per the manufacturer's recommendations (ANALGESIC TREATMENT; AT). In terms of behavioural expression, it was expected that: CONTROL ≠ PLACEBO, AT = CONTROL, and PLACEBO ≠ AT. Video footage of the 6−8-week-old lambs (*n* = 10 for each treatment) was captured approximately 1.5 h post-procedure and was presented, in a random order, to 19 observers for assessment using the Free-Choice Profiling (FCP) approach to QBA. There was significant consensus ($P < 0.001$) among the observers in their assessment of the lambs, with two main dimensions of behavioural expression explaining 69.2% of the variation. As expected, observers perceived differences in the demeanour of lambs in the first dimension, scoring all lambs subject to the routine husbandry procedures as significantly more *'dull'* and *'uneasy'* compared to the control lambs ($P < 0.05$). Contrary to expectations, the results also suggested that analgesic treatment did not provide relief at the time of observation. Further investigations to validate the relationship between behavioural expression scores and pain are necessary, but these results suggest that painful husbandry procedures alter the behavioural expression of lambs and these differences can be captured using QBA methodology.

5.2 INTRODUCTION

Animal species differ markedly in their observable reactions to tissue damage: some display clear signs of distress (e.g., pigs), while others (e.g., sheep) show little, if any, overt behavioural responses (Broom, 1998), even after surgical procedures that have been shown to be painful and aversive (Rushen and Congdon, 1987). The assessment of pain in stoic animals such as sheep, therefore, can be particularly challenging (Dwyer, 2004; Fitzpatrick et al., 2006). Moreover, for painful husbandry practices such as tail docking, castration, ear tagging, and mulesing in lambs, the evaluation of the efficacy of pain relief may also be made difficult by the sheep's stoic nature (McLennan, 2018). As such, it is clear that objective measures of pain in sheep to recognise and quantify pain are needed.

Numerous studies have investigated the physiological and behavioural responses of lambs to common painful procedures (Molony and Kent, 1997; Thornton and Waterman-Pearson, 1999; Grant, 2004; Karakuş and Karakuş, 2017). However, the results of these studies have been inconsistent and sometimes contradictory, and the success of the measures in identifying and quantifying different types of potential pain-inducing scenarios, variable. For example, the adoption of abnormal standing postures in lambs following castration found by Molony et al. (1993), was not evident in the research by Grant (2004). Likewise, tail wagging and kicking/foot stamping have proved useful (Kent et al., 2000; Molony et al., 2002) and not useful (Grant, 2004; Colditz et al., 2012) for the identification of pain in lambs after castration. Furthermore, standing still (statue standing) is common in lambs following surgical castration (Molony et al., 1993; Molony et al., 2002), but not in lambs castrated with rubber rings (Molony et al., 1993), or in lambs that have been tail docked (Marchewka et al., 2016). These inconsistencies pose a significant problem for the management of sheep as they may limit a producers' motivation to use analgesics (Larrondo et al., 2018). Pain relief in livestock is not only important to achieve good animal welfare, but for producers to meet societal expectations of the duty of care, which is essential to protect their social licence to farm.

It has been suggested that qualitative observation by experienced personnel is perhaps one of the best methods for capturing the complexity of pain in animals (Rutherford, 2002; Lizarraga and

Chambers, 2012). Indeed, qualitative assessments and observations are commonly used in veterinary fields and human medicine, with informal qualitative observations tending to accompany quantitative results within pain-related research (Wemelsfelder and Farish, 2004). Qualitative behavioural assessment (QBA) is a ‘whole animal’ approach that is proposed to capture the dynamic body language of animals (demeanour or behavioural expression behaviour), through the integration and summary of details of behavioural events, posture, and movement (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001; Wemelsfelder and Lawrence, 2001). Several studies, in various livestock species, have supported QBA, indicating significant associations with standard physiological and behavioural measurements pertinent to the assessment of sheep welfare (Fleming et al., 2016). Perhaps more importantly, there is evidence demonstrating the usefulness of QBA in the study of animal pain. Indeed, QBA has been reported to identify differences in behavioural expression in animals suffering injury and presumably painful disease (skin lesions (Camerlink et al., 2016), mastitis (de Boyer des Roches et al., 2018), lameness (Grant et al., 2018), and flystrike (Grant et al., 2019)), and painful procedures (Vindevoghel et al., 2019). The integrated, dynamic nature of this approach may capture the complex behaviour of lambs following painful routine husbandry procedures such as mulesing and tail docking.

The gold-standard for validating behavioural responses to pain involves the evaluation of responses with and without analgesic treatments (Weary et al., 2006). The development and adoption of analgesic treatments for those procedures that cause pain in lambs, particularly mulesing (i.e., the surgical removal of strips of wool-bearing skin around the perineum and the tail to prevent flystrike, or cutaneous myiasis), has received significant attention in recent years (Paull et al., 2007; Lomax et al., 2008; 2009; 2013). Advice for the practical management of pain includes the application of the topical analgesic Tri-Solfen (Windsor et al., 2016). Further, there is evidence to suggest that the additional application of nonsteroidal anti-inflammatory drugs (NSAIDs) may provide longer and/or more pronounced pain relief in lambs (Paull et al., 2007; Small et al., 2018a; Inglis et al., 2019). The present study was part of a larger study that investigated the behavioural expression of lambs under field conditions following routine procedures performed at lamb marking (ear tagging, castration, mulesing, and tail docking), with and without the application of analgesic treatment (Tri-Solfen and an NSAID, meloxicam) (Inglis et al., 2019). We hypothesised that observers using QBA methodology would be able to differentiate between the behavioural expression of lambs undergoing painful procedures, with and without analgesia, and those that only had the procedural restraint. If the analgesic protocol provided adequate pain relief then it was expected that the lambs that received analgesia would display similar behaviour to those that had just been restrained, whereas the lambs that did not receive analgesia would display more pain-related behaviour.

5.3 MATERIALS AND METHODS

The Animal and Human Ethics Committees at Murdoch University (R2903/17; R2598/13; O2780/15; 2008/021) approved this study, thus ensuring compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, the Australian Code for the Responsible Conduct of Research 2007, and the National Statement on Ethical Conduct in Human Research, 2007. The study was performed at Murdoch University’s Whitby Falls farm in Whitby, Western Australia (Latitude: -32.293056; Longitude: 116.015278) in July 2017.

5.3.1 Animals and experimental design

The behaviour of 30, 6–8-week-old, mixed-sex (15 male and 15 female) Merino lambs was assessed from video footage collected in the paddock. The animals used in this study formed part of a larger project designed to test the efficacy of the NSAID meloxicam (Metacam 20, Ilium, Troy Laboratories Pty Ltd., Australia) in mitigating pain in lambs following ear marking, castration, tail docking, and mulesing, where other behavioural measurements were taken and other combinations of analgesics were applied (Inglis et al., 2019). In brief, the lambs used were allocated to treatments based on live-weight, sex, and rear type (number of lambs reared per ewe), and were housed in adjacent pens (50 m × 50 m) with their dams. All lambs were identified by numbers spray-painted on their sides, with additional unique marks on their head, limbs, or body. Any routine husbandry procedures: ear marking, castration using rubber rings, tail docking using a gas-heated knife, and surgical mulesing; were performed in a specially designed ‘marking cradle’ (LC264, Harvestaire Pty Ltd., Perth, Australia) by a Livestock Association of Australia accredited contractor, and all lambs (except the CONTROL group) were also vaccinated against scabby mouth (ScabiGard, Zoetis Australia Ltd. , Sydney, Australia), and cheesy gland and clostridial diseases (Glanvac, Zoetis Australia Ltd., Sydney, Australia), with females also vaccinated against Johne's disease (Gudair, Zoetis Australia Ltd., Sydney, Australia).

The 30 lambs used in the present study were randomly selected from 3 of 7 treatments imposed by Inglis et al. (2019):

(i) CONTROL lambs were held in the cradle for 60 sec but did not undergo the husbandry procedures (n = 10).

(ii) PLACEBO lambs were given subcutaneous saline 15 min prior to the husbandry procedures (n = 10).

(iii) ANALGESIC TREATED (AT) lambs were given subcutaneous meloxicam (1 mg/kg,) 15 min prior to the husbandry procedures in the cradle. This was immediately followed by application of a topical analgesic (Tri-Solfen, Lignocaine 40.6 g/L, adrenaline 24.8 mg/L, bupivacaine 4.5 g/L, cetrimide 5 g/L, Bayer Australia Ltd., Sydney, Australia) to the mulesed area and tail-docking wound (8–10 mL based on lamb weight; see Inglis et al. (2019)). Analgesic agents were administered using manufacturer's recommendations (n = 10).

5.3.2 Qualitative behavioural assessment (QBA)

5.3.2.1 Observers and collection of video footage

The QBA assessment videos were made up of 30 videos clips (one per lamb), with footage of lambs from the three experimental treatments: CONTROL, PLACEBO and AT collected in the paddock approximately 1 h 32 min (± 25 min) post-procedure. Lambs were filmed at a distance (outside the bounds of the pens) using two hand-held video cameras (Panasonic HC-W570M: Panasonic Corporation, Kadoma, Japan) and footage was on average 29 ± 5 s in length. Footage was selected and edited to ensure evidence of husbandry procedures were not visible to QBA observers in either the assessment clips or in the term generation session in the Free-Choice Profiling (FCP) procedure.

Consequently, observers viewed the lambs only from the front and side, so they were blind to both possible analgesic treatment and the status of the lambs regarding the husbandry procedures. However, observers were unavoidably aware of ear marking status.

Nineteen observers from Murdoch University students and staff (16 female and 3 male) were recruited to assess lamb behaviour using the FCP approach to the QBA methodology. These observers were recruited by advertising via email, flyers around the University campus, and on social media, with all those that responded accepted into the study. Of these 19 observers, only 3 (15.8%) could be classified as experienced with sheep, whereas the remaining 16 (84.2%) indicated that they had limited or no previous experience with sheep.

5.3.2.2 Free-choice profiling procedure

To complete the QBA by means of the FCP procedure, observers were required to generate their own lists of descriptive terms, and then score the behavioural expression of lambs using these. To generate terms, observers were shown a series of video clips of sheep, both lambs and adults, demonstrating a range of behaviours (*n* = 12). After watching each of these clips, observers were asked to list terms they thought described the animals' behavioural expressions. Although observers had only 2 min after each clip to write down their terms, no limits were imposed on the number of these they could use. After all videos were watched, these lists were edited to remove terms that described actions, and for ease of scoring, negative terms were transformed to their stem word (e.g. *'uncomfortable'* became *'comfortable'*). The result was a unique, randomly ordered, list of descriptive terms for each observer. Each descriptive term in these lists was then attached to a visual analogue scale (VAS; minimum to maximum) in an electronic worksheet (Microsoft Excel 2003, North Ryde, NSW, Australia). The observers then used their own unique list of descriptive terms to score the full set of randomly ordered lamb videos (*n* = 30). With each term attached to a VAS, observers were instructed to score each animal's behavioural expression, where their mark on the scale between minimum and maximum reflected the intensity of each animal's expression of that term. Observers viewed the assessment clips independently and did not have the opportunity to confer with each other.

5.3.3 Statistical analysis

For QBA, the distance from the minimum point of the VAS to where the observer had made a mark for each term was calculated (where minimum = 0 and maximum = 100), and these observer scores were analysed by means of Generalised Procrustes Analysis (GPA) (Genstat 2008, VSN International, Hemel Hempsteat, UK; Wemelsfelder et al. (2000)). See Wemelsfelder et al. (2000); Wemelsfelder et al. (2001) for further details concerning these methods, including a detailed description of GPA analysis and output interpretation procedures.

Each of the assessment lambs received a score on the two main GPA consensus dimensions and these scores were BoxCox-transformed to conform to the requirements of parametric statistics with visual confirmation of residuals. A mixed-model analysis of variance (ANOVA) (Statistica 7.1, StatSoft-Inc., North Melbourne, Vic., Australia) was performed to determine if there was an effect of treatment (fixed factor) on the transformed GPA dimension scores given to the lambs on dimensions 1 and 2 (dependent variables), with clip ID (animal) included as a random factor. Post hoc pairwise ANOVAs comparing the GPA dimension scores between each treatment group (fixed factor) were performed with clip ID (animal) included as a random factor. To further investigate interobserver reliability, the GPA scores each lamb received from the 19 observers were correlated using Kendall's coefficient of concordance W.

5.4 RESULTS

The 19 observers using the FCP methodology generated a total of 74 unique terms to describe the lambs they were shown (average 15.2 ± 4.4 terms per observer; range 8–25). The GPA consensus profile explained 53.7% of the variation between observer scores of lambs and this differed significantly from the mean randomised profile (t99 = 60.7, $P < 0.001$). Two main dimensions of behavioural expression were identified, explaining 51.4% and 17.8% of the variation in scores given to individual lambs for GPA dimensions 1 and 2, respectively. The moderate level of agreement from the 19 observers in this group regarding the perceived behavioural expression scores of lambs is reflected by the reported W values of 0.66 and 0.54 on GPA dimensions 1 and 2, respectively ($P < 0.001$; Table 5.1).

Although 4 observers fell outside the 95% confidence region, removal of these did not stop the consensus from being highly significant or alter treatment effects, nor did they appear to belong to any specific demographic group, and thus were retained in this study.

The word charts generated for each of the observers appeared to be semantically consistent, with terms converging towards similar meanings. The terms from all observers were pooled and those with the strongest loadings (positive and negative) for each of the GPA dimensions are shown in Table 5.1. Terms such as *'happy'* and *'focused'* were associated with low values for GPA dimension 1, whereas terms such as *'dull'* and *'uneasy'* were associated with high values on this same dimension. For GPA dimension 2, terms such as *'dazed'* and *'docile'* were associated with low values, and terms such as *'curious'* and *'inquisitive'* with high values. The two most frequently used terms for each GPA dimension, as shown in Table 5.1, were selected for the purpose of describing and labelling the GPA dimensions in relation to the experimental treatment group (Figure 5.1).

Table 5.1. Terms used by observers to describe the behavioural expression of lambs filmed in the paddock following routine painful husbandry practices (ear tagging, castration, mulesing and tail docking). Those terms that had strong loadings with the two main Generalised Procrustes Analysis (GPA) dimensions are listed. Terms with loadings > 0.6 (high values) and < -0.6 (low values) are displayed for GPA dimension 1, and for GPA dimension 2, those with loadings > 0.4 (high values) and < -0.4 (low values). Term order is determined first by the number of observers that used each term to assess lamb behaviour (with numbers presented in parentheses), and second by the loading of that term.

GPA dimension (% of variation explained) Kendall's *W* score	**Low values**	**High values**
GPA 1 (51.4%) $W = 0.66^{***}$	Happy (15), focused (8), sure (5), confident (4), motivated (1), lively (1), certain (1), at ease (1), active (1), purposeful (1), bright (1), perky (1).	Dull (5), uneasy (1), weary (1), tentative (1), sluggish (1).
GPA 2 (17.8%) $W = 0.54^{***}$	Dazed (2), docile (2), secure (1), sluggish (1), weary (1).	Curious (12), inquisitive (1), lost (1), skittish (1), purposeful (1), restless (1).

Overall, there were significant treatment effects on the first GPA dimension ($F_{2, 27} = 6.25$, P = 0.006), but not the second ($F_{2, 27} = 0.39$, $P = 0.68$; Figure 5.1). GPA dimension 1 scores for the lambs in the CONTROL treatment were significantly lower than those from the AT ($F_{1, 18} = 12.82$, $P = 0.002$) and the PLACEBO groups ($F_{1, 18} = 6.41$, $P = 0.021$), with the observers scoring the CONTROL lambs as more *'happy'* and *'focused'* compared to those lambs that were subject to the painful husbandry procedures both with and without analgesic treatment. There were, however, no differences between the observer scores of the lambs in the PLACEBO and AT treatments ($F_{1, 18} = 1.06$, $P = 0.32$) on this dimension. In addition, there were significant differences in GPA scores between clips (animals) on this dimension ($F_{27, 540} = 23.43$, $P < 0.001$; Figure 5.2) and GPA Dimension 2 ($F_{27, 540} = 22.96$, $P < 0.001$; Figure 5.2).

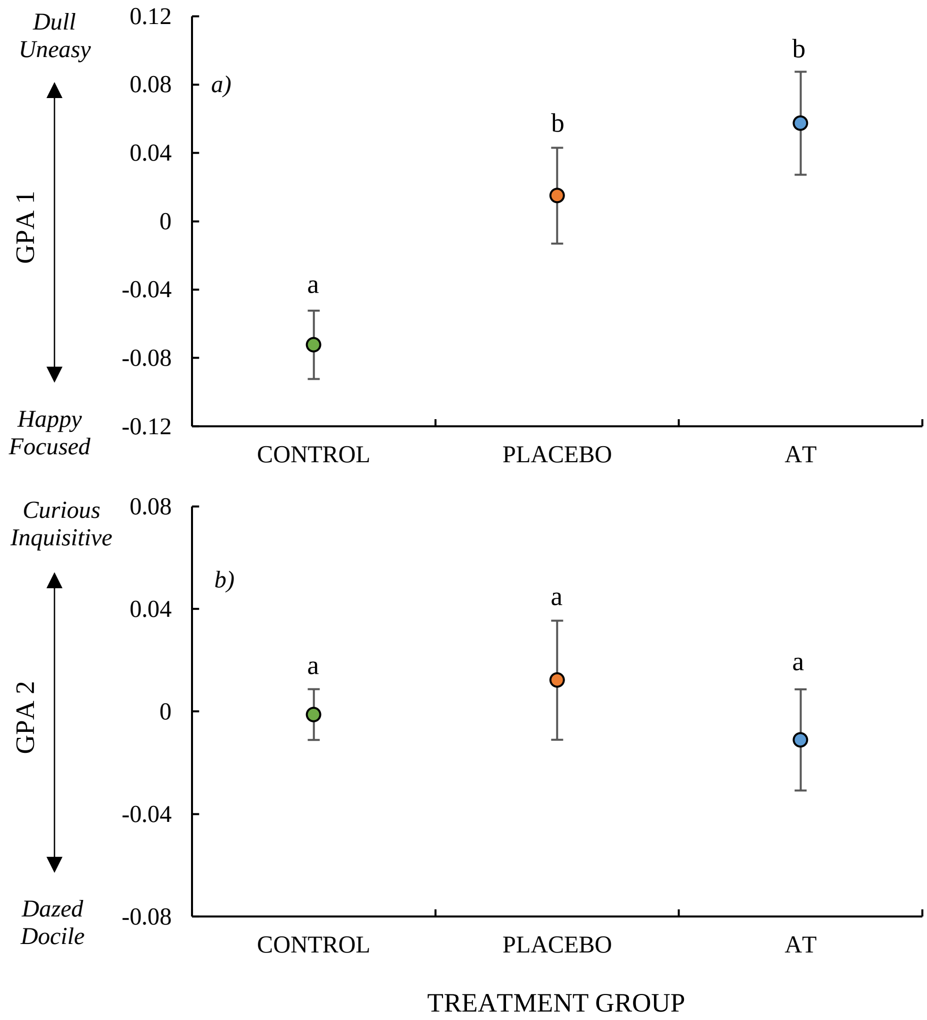

Figure 5.1. Effects of the treatment groups: CONTROL (green marker; *n* = 10), PLACEBO (orange marker; *n* = 10) and ANALGESIC TREATMENT (AT) (blue marker; *n* = 10) on General Procrustes Analysis (GPA) scores on Dimension (a) 1 and (b) 2, assessed from video footage taken of lambs in the paddock approximately 1.5 h post-procedure. Different letters indicate significant differences between treatment groups.

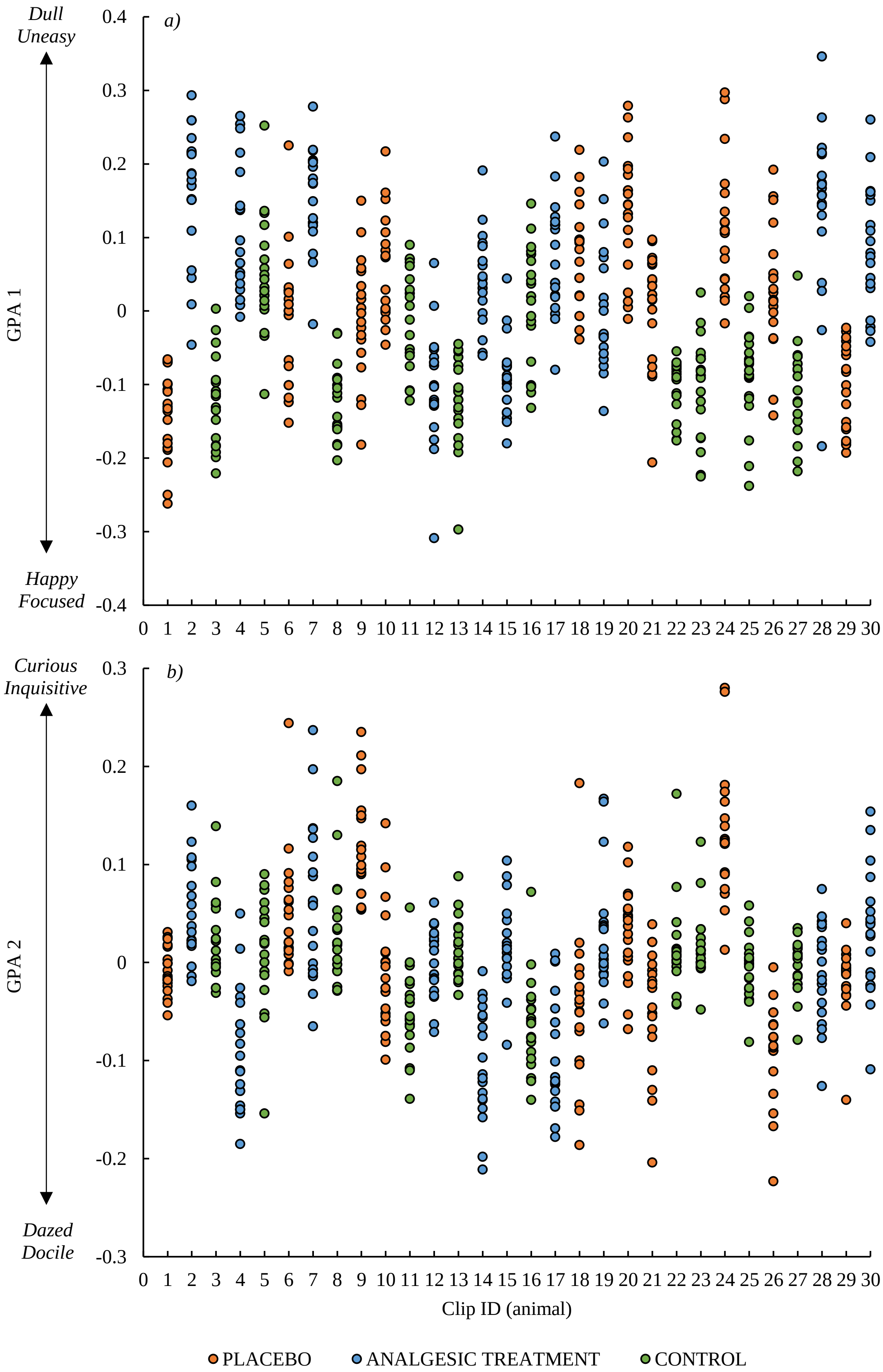

Figure 5.2. Individual observer General Procrustes Analysis (GPA) scores given to each of the 30 clips (animals) on GPA Dimension (a) 1 and (b) 2.

5.5 DISCUSSION

To evaluate the validity of the QBA methodology to assess pain in lambs, their behavioural expression in response to routine husbandry procedures performed at lamb marking (ear tagging, castration, mulesing, and tail docking), with and without analgesic treatment, was investigated. In the present study, observers perceived a significant difference between the demeanour of CONTROL lambs and those that were subject to the painful husbandry procedures, describing the CONTROL lambs as significantly more *'happy'* and *'focused'* compared to both the PLACEBO and AT lambs (more *'dull'* and *'uneasy'*). Furthermore, contrary to expectation, at 1.5 h post-procedure the behavioural expression of the lambs that received the analgesics Tri-Solfen and meloxicam (AT) was not different to that of the lambs that were given a placebo treatment with saline (PLACEBO). These results align with those of the overarching study (Inglis et al., 2019) and suggest that not only do these husbandry procedures alter the behavioural expression of the lambs as expected, but that the administration of analgesics failed to normalise scores of behaviour expression in AT lambs. The latter of which implies that the analgesics provided (Tri-Solfen and meloxicam) did not ameliorate pain in lambs 1.5 h after the procedures. Given that the observers both reached a significant consensus in their assessments of behavioural expression of lambs, and identified a difference in demeanour seemingly related to the expression of pain in the AT and PLACEBO lambs, this study offers support for the use of the QBA methodology to identify the expression of pain in lambs under field conditions. Although these results are encouraging, this study represents the first step in the validation process and work is needed to verify these responses with the use of appropriate analgesics which are the gold standard (Weary et al., 2006).

In the present study, observers described and scored the behavioural expression of all animals that were subject to the painful husbandry procedures, regardless of whether they received analgesic treatment or not (AT or PLACEBO), as more *'dull'* and *'uneasy'* compared to the control lambs that were only restrained in the cradle for 60 s. It is undeniable that the mulesing procedure causes pain as a result of severe tissue damage and ensuing inflammatory responses, with the behavioural response to the procedure one of 'shock', characterised by reduced activity and the adoption of an abnormal 'hunched' posture while standing, with increased sensitivity to stimulation (Fell and Shutt, 1989;

Chapman et al., 1994; Grant, 2004; Paull et al., 2008; Edwards et al., 2011; Lomax et al., 2013). Furthermore, the addition of tail docking and/or castration with rubber rings at the time of mulesing in the present study is likely to have also altered the behavioural expression of lambs in a way that was evident to observers, since the combination of these procedures is known to increase the expression of active pain avoidance behaviours in lambs (Grant, 2004). Thus, it is likely that the differences observed between the CONTROL lambs and the PLACEBO and AT lambs reflects the disruption of behaviour caused by these procedures. Given that the larger overarching study reported fewer normal behaviours and more pain-related behaviours in placebo lambs compared to control lambs from 1 h post-procedure, and no difference in the behaviour of the AT lambs compared to the PLACEBO and CONTROL animals until 2 h post-procedure (Inglis et al., 2019), it was not unexpected that lambs subject to these painful procedures in the present study would display behavioural patterns and demeanours different to that of the control animals at 1.5 h post-procedure and that these differences would be evident to the observers using the QBA methodology. Although preliminary in nature, these results are promising and suggest that QBA may provide useful and meaningful information related to the response of lambs to severe pain caused by routine husbandry procedures performed at lamb marking. Thus, following further development and validation, QBA may become a tool to aid producers in identifying animals that are in pain, and to determine whether an animal has received sufficient analgesia, which will ultimately improve welfare.

Contrary to expectation, the administration of analgesics in the present study did not alter the behavioural expression scores between the AT and PLACEBO lambs. This lack of discernible difference in behavioural expression between the AT and PLACEBO lambs may suggest that the analgesic protocol used in the present (and the overarching) study had not yet eliminated the pain caused by the painful husbandry procedures at the time of assessment. This is an animal welfare concern that warrants urgent investigation. If this is the case, then we cannot expect our hypothesised responses (CONTROL ≠ PLACEBO, AT = CONTROL and PLACEBO ≠ AT) to hold true; rather, as was found, it is expected that AT and PLACEBO lambs would display similar behavioural responses, and that the behaviour of lambs in both of these groups would be different from that of the CONTROL lambs. Such patterns are consistent with those of previous studies in which an analgesic treatment was not effective at reducing the responses of lambs to mulesing (e.g. Paull et al., 2008; Small et al., 2018a). Furthermore, the absence of a discernible analgesic effect on behavioural expression between AT and PLACEBO lambs at 1.5 h post-procedure in the present study align with those results of the larger overarching study, where differences in pain and lying behaviours between the equivalent AT and PLACEBO lambs only became evident from 4 h post-procedure, and differences did not appear at all in the amount of normal behaviours recorded within the first 6 h post-procedure (Inglis et al., 2019). Thus, it is likely that the analgesic protocol did not reduce the pain experienced by the AT lambs in the present study, and hence explains the lack of difference in behavioural expression between the AT and PLACEBO lambs 1.5 h post-procedure. As proposed in the overarching study (Inglis et al., 2019), perhaps this lack of analgesic effect was, at least in part, a consequence of suboptimal dose rate or time of administration of meloxicam and/or Tri-Solfen. This is an issue that has also been raised by Paull et al. (2008) regarding the administration of the NSAIDs meloxicam and tolfenamic acid following surgical mulesing in lambs. Alternatively, perhaps the effectiveness of the analgesic treatment to control the pain experienced by the lambs was reduced in the present (and the overarching) study since the lambs were subject to multiple procedures, and these procedures are reported to cause different types and levels of pain (Grant, 2004). For example, surgical mulesing causes significant skin tissue damage, whereas castration with rubber rings causes ischaemic pain, with each procedure evoking different responses in lambs (Grant, 2004). More work is needed to verify these results and to improve our understanding regarding the impact appropriate analgesic treatment has on the behavioural expression of lambs. In particular, a more detailed behavioural and physiological assessment of lambs following painful husbandry procedures together with the evaluation of the behavioural expression of lambs beyond 2 h post-procedure would prove valuable in this regard.

The failure of the analgesic protocol to alter the behavioural expression of the AT lambs compared with the PLACEBO lambs could be considered unexpected; however, it is evident that there are inconsistencies throughout the literature concerning the onset of ameliorative effects of analgesic agents in animals following these husbandry procedures. For example, whether used singly or in combination with NSAIDs, Tri-Solfen is reported to provide rapid onset analgesia in mulesed lambs as evidenced by reductions in lamb cortisol responses, decreased behavioural indicators of pain, and significant wound anaesthesia within the first hour following mulesing and application (Paull et al., 2007; Lomax et al., 2008; 2013). However, there is also evidence that suggests behavioural differences between lambs mulesed with or without pain relief that incorporates Tri-Solfen and/or meloxicam (buccal or subcutaneous) only become evident from 2–4 h post-procedure (Small et al., 2018b; Inglis et al., 2019). In this situation, the assessment of animals beyond 2 h post-procedure is necessary, not simply because the verification of behavioural measures with the administration of appropriate pain relief is the gold standard for the validation of measures (Weary et al., 2006), but because it is important from a welfare perspective to confirm the efficacy and coverage that currently available analgesics offer animals. Currently, the adoption of the best-practice pain management by producers is particularly important as society expects animals raised in production industries to be treated humanely and to receive high levels of care (Blokhuis et al., 2003). To avoid public criticism and to protect their social licence to farm, producers must take actions to meet such expectations, and this involves the application of analgesics that provide rapid and consistent amelioration of pain, particularly in instances where pain is caused by human intervention. The challenge is now to determine the most effective analgesic protocols to protect lambs from the pain caused by those routine husbandry procedures performed at marking.

The identification and assessment of pain in animals are complex (Landa, 2012), and may be difficult in lambs, given their behavioural responses can be subtle and conflicting (Lomax et al., 2008). Though perhaps difficult, we are obliged as a humane society to give these animals the benefit of the doubt, and in the absence of evidence that demonstrates they are not sentient, to accept that there is a moral imperative to treat them as sentient. While independent animal welfare science is critical to the debate, so too are societal morals and ethics, which shape the ‘social licence’ that permits animal use. That the observers in the present study were able to distinguish AT and PLACEBO lambs from the CONTROL lambs suggests that QBA methodology is useful in the detection of pain by allowing observers to interpret and integrate various behavioural and postural signs into a simple yet meaningful assessment. Still, it is important to note that these observed differences in the behavioural expression may not have been caused solely by the pain of the husbandry procedures the lambs underwent. For example, lambs may have had an adverse reaction to one of the vaccines administered or the ear tagging may have caused discomfort or irritation that altered or disrupted the behaviour of these lambs in a way that was perceived by the observers. Additionally, the potential impact factors such as the small sample size, inexperienced observers, and short length of observations, should also be considered in the interpretation of the results. For example, given Tri-Solfen is reported to reduce behavioural and physiological indicators of pain within 1 h post-treatment (Paull et al., 2007; Lomax et al., 2008; 2013), it could be that the analgesic protocol administered in the overarching study provided at least some relief to the AT lambs, but that any differences in the behavioural responses of these lambs and those in the PLACEBO group were too subtle or difficult to characterise at 1.5 h post-procedure, or that the observers did not have the experience to recognise and distinguish between different pain responses, only between lambs that were in pain and those that were not. Indeed, while there is evidence that the observers' level of experience with the focal species does not weaken QBA assessments (Napolitano et al., 2012; Wemelsfelder et al., 2012; Phythian et al., 2013a), there is also evidence to the contrary (Gronqvist et al., 2017). There is also some evidence that observer gender may influence behavioural assessments (Marsh and Hanlon, 2004) and given 84% of the observers in the present study were female, this is a factor that future studies should explore. It is also possible that the lambs were not observed for long enough for further potential differences (e.g., AT vs. PLACEBO) to become evident to observers using the QBA methodology. While there is evidence that observers can distinguish differences in sheep behavioural expression from videos of shorter duration (Wickham et al., 2015; Serrapica et al., 2017; Grant et al., 2018; Grant et al., 2019), these studies do not explicitly focus on the assessment of pain and analgesic treatment. Indeed, the only other study involving the assessment of pain and analgesic treatment in livestock using QBA presented observers with 40–50 s of video footage per animal (Vindevoghel et al., 2019), compared to the 30 s in the present study. As such, to further validate the QBA methodology and to better understand the behavioural response of lambs to pain, efforts should be made to address these concerns. In particular, there is an obvious need to investigate the behavioural expression of a larger sample of lambs beyond 2 h post-procedure. Studies that utilise experts or at least persons more experienced than those used herein, are also necessary, particularly since those that are likely to use the approach (e.g., producers and stock persons) are also likely to be familiar with both sheep and the assessment of pain. In addition, comparison in the use of inexperienced vs. experienced observers for the assessment of pain in lambs may be insightful. Lastly, a comparison of the behavioural expression between lambs pre- and post-surgery and analgesic treatment such as that completed by Vindevoghel et al. (2019) in their evaluation of the pain response of cattle to castration, could prove valuable.

5.6 CONCLUSIONS

This study is the first step in investigating the validity of the QBA methodology to assess pain in lambs following those husbandry procedures performed at marking. That the observers identified differences in demeanour between CONTROL lambs and those that were subject to painful husbandry procedures and were either administered saline (PLACEBO) or analgesics (AT), strongly suggests that these procedures cause pain and that this pain alters the behavioural patterns and demeanour of lambs in a way that can be captured using the QBA methodology. Although further work is needed to verify the behavioural expression of lambs following these painful husbandry procedures, and to validate these responses to the gold standard with the use of an effective analgesic protocol, these results suggest that QBA could be a valuable tool to aid producers in the recognition and management of pain in lambs. As the results of this study and others (Small et al., 2018b; Inglis et al., 2019) suggest that the use of Tri-Solfen, even in conjunction with meloxicam, may not provide effective pain relief in lambs within the first 2 h following procedures such as mulesing and tail docking that are performed at marking, it is advised that further studies are urgently needed to investigate and rectify this potential welfare gap. This study is the first of its kind in sheep and highlights several avenues for future work needed to validate QBA methodology to assess pain in this species. However, results are encouraging and demonstrate the potential for producers to use QBA to identify and manage pain in lambs. This will not only improve the welfare of lambs undergoing painful husbandry procedures, such as mulesing, but will assist the social licence for sheep farming in Australia.

CHAPTER 6 WHAT CAN THE QUANTITATIVE AND QUALITATIVE BEHAVIOURAL ASSESSMENT OF VIDEOS OF SHEEP MOVING THROUGH AN AUTONOMOUS DATA CAPTURE SYSTEM TELL US ABOUT WELFARE?

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6.1 ABSTRACT

Sheep can be exposed to a variety of challenges and failure to adapt to these challenges can compromise their health and wellbeing. Regular monitoring of stock on large-scale or extensive systems may not always be possible, although recent technological advancements in automated data capture, such as walk-over-weighing (WoW), can make monitoring easier. The potential benefit of including behavioural assessment in such a system has yet to be tested. We investigated whether quantitative and qualitative behavioural assessment (QBA) methods could be applied to short video footage collected automatically within a WoW setup, to differentiate between sheep that were, presumably, in different (positive and negative) welfare states. Video footage was collected from 36 Merino sheep within the four treatment groups; Control (n = 12), Habituated to the WoW setup and human interaction (n = 8), Lame (n = 8) and Inappetent (n = 8). Habituated sheep were exposed to a low-stress handling regime for six consecutive days prior to filming. At the same time, feeding behaviour was recorded by means of radio-frequency identification (RFID) technology to identify sheep suffering inappetence. Lame sheep were identified using a 6-point scoring system, and Control animals were selected ensuring that they were not Lame, Inappetent or Habituated. For QBA, the footage was presented, in a random order, to 18 observers. There was a significant ($P < 0.001$) consensus among the observers in their assessment of the behavioural expression of the sheep. Observers described the Habituated and Lame sheep as significantly more *'focused/collected/assured'* compared to the Control sheep ($P < 0.05$). There was no difference in observer scores between the Inappetent sheep compared to the Controls. A number of associations were found between the QBA scores and the quantitative behavioural measures recorded. Sheep that baulked more frequently at the entrance to the WoW system ($R_s = -0.70$; $P < 0.001$) or had a greater number of circling events ($R_s = -0.68$; $P < 0.001$) were described as more *'reluctant/tense/wary'*, while those that recorded faster walking speeds ($R_s = 0.65$; $P < 0.001$) or spent less time standing stationary ($R_s = -0.48$; $P < 0.01$) were described as more *'focused/collected/assured'*. We conclude that qualitative and quantitative behavioural measures can be used to identify differences in animal behaviour, presumably related to their welfare state, when applied to short video clips automatically collected in a WoW setup. These findings suggest that behavioural measures could be

collected, practically, within automated biometric data capture systems to provide additional information to aid in the assessment of sheep welfare in extensive systems.

6.2 INTRODUCTION

The health and wellbeing of sheep can be subjected to a range of challenges within their production environments, not only from routine husbandry procedures but also changes in management, social structure or environmental conditions (Hargreaves and Hutson, 1990b; Wemelsfelder and Birke, 1997). The failure of sheep to adapt to these challenges can result in compromised health, reduced production, and economic losses (Lynch et al., 1992; Barnes et al., 2008; Rice et al., 2016). Producers are under increasing pressure from animal welfare groups to allocate more time and labour resources to monitor sheep welfare (Morris et al., 2012); however, larger-scale enterprises covering vast areas, and with limited labour and infrastructure inputs, have found it difficult to answer these demands (Petherick, 2006).

New technologies, such as animal radio-frequency identification (RFID) tags and automated biometric data capture, may allow for more efficient and cost-effective monitoring in both extensive and intensive sheep management systems. An automated biometric data capture technology used commercially is walk-over-weighing (WoW). In this system, livestock are trained to walk through a passageway containing weighing scales to gain a reward (e.g. access to water). As the animals walk through the passageway, an electronic ear tag reading panel records their identity along with the time and date of the event. Producers can thus identify live-weight changes, which may reflect changing states of animal health and welfare. Live-weight change alone cannot reflect the variety of welfare problems present in production environments, and collection of behavioural measures of animal welfare would, therefore, be beneficial if incorporated into automatic biometric data capture systems. Indeed, the success of the Pedigree MatchMaker system to match parentage in sheep with a similar success to DNA parentage testing (Kemmis et al., 2016), represents a strong example of how existing biometric data capture systems can be adapted to provide meaningful behavioural information. Much like the WoW system, this system utilises an electronic panel reader system, that records sheep movement data to identify associations between lambs and dams with the purpose of determining parentage (Richards and Atkins, 2007).

Visual assessments of animal behaviour can provide meaningful indicators of welfare and are commonly employed in many production industries (Mench and Mason, 1997; Webster, 2005a). Indeed, most livestock producers would say that they find it reasonably easy to visually identify a sick sheep by the way the sheep stands, moves or interacts with conspecifics. Scientists call this ‘behavioural expression’, but we could also talk about ‘body language’ or ‘demeanour’ (Wemelsfelder et al., 2012). Body language reflects not only the animal’s physical or physiological state but potentially also its psychological (emotional or affective) state (Boissy et al., 2007; Rutherford et al., 2012; Murphy et al., 2014). Qualitative Behavioural Assessment (QBA) is a methodological approach for capturing the body language of animals in numerical terms that can then be analysed statistically (Fleming et al., 2016). QBA has been supported by many studies demonstrating significant associations with standard behavioural and physiological measurements relevant to welfare assessment (reviewed by Fleming et al., 2016). QBA is suited for on-farm application, being quick, easy to implement and non-invasive, as it simply relies on observers watching live or previously-captured video camera footage of the animals (Wemelsfelder, 1997; Wemelsfelder et al., 2000; Wemelsfelder and Lawrence, 2001; Boissy et al., 2007). Thus, QBA could be used with footage collected by a video camera system incorporated into an automated biometric data capture setup, such as WoW.

To date, few comprehensive protocols that target the assessment of both positive and negative welfare conditions in animals, on-farm, have been established. One of these, the 2004-2009 European Commission’s Welfare Quality ® audit (European Union, 2011), captured positive aspects of welfare in cattle employing the QBA methodology (Keeling et al., 2013). The successful identification of signs of positive welfare on-farm would provide an additional assessment that would extend the value of on-farm monitoring of welfare for farm animal management guidelines (Farm Animal Welfare Council, 2009; Webster, 2011; Edgar et al., 2013).

We hypothesised that QBA and quantitative behavioural measures could be applied to short video camera footage, collected automatically within a WoW setup, to differentiate between sheep that were selected based on differing conditions of presumed welfare, both positive (+ve) and negative (-ve), i.e. (1) inanition (-ve: Barnes et al., 2008; Besier et al., 2010); (2) lameness (-ve: Lynch et al., 1992;

Goddard et al., 2006); and (3) habituated to WoW setup and human interaction (+ve: Manteuffel et al., 2009).

6.3 MATERIALS AND METHODS

These experiments were approved by the Animal and Human Ethics Committees at Murdoch University (R2598/13; O2780/15; 2008/021) to ensure compliance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, the Australian Code for the Responsible Conduct of Research 2007, and the National Statement on Ethical Conduct in Human Research, 2007. The experiment was conducted at a private sheep property, Wellard La Bergerie feedlot, located in Mundijong, Western Australia (WA) (Latitude: 32.3º S; Longitude: 116.0º E).

6.3.1 Animals and housing

All sheep used in the study were selected from a source population of 877 one-year-old Merino wethers (castrated males) that had arrived at the feedlot from several farms within the South-West region of WA. All sheep used in the study were housed in three raised feedlot pens (about 270 - 300 sheep per pen), with dimensions of 10 x 25 m, on mesh floors that were under cover with solid sides 0.7 m high and then open to the roof. All sheep had ad libitum access to clean water and pelletised feed, with feed and water troughs refilling automatically.

6.3.2 Experimental groups

Sheep were observed over a week to identify individuals that represented each of the four treatment groups, which were then filmed on Day 7 after arrival at the feedlot.

6.3.2.1 Inappetent group

Inanition, or the persistent and voluntary refusal to eat, causes a significant negative welfare state in sheep (Barnes et al., 2008; Besier et al., 2010). All 877 sheep in the study were fitted with RFID ear tags upon arrival at the feedlot (Day 0). The feed troughs were fitted with antennae to detect the RFID tags when the sheep had their heads in the trough. A feeding session was allocated to a sheep when their RFID tag was within range of the antennae for more than 5 seconds, and the total time and number of visits to the feed troughs was recorded. The feed trough attendance data was collected over six consecutive 24-hour periods, and the frequency distribution of daily feed trough attendance was used to select animals for the Inappetent group. Eight sheep were selected as being in a state of inanition, based on trough attendance data indicating that an individual's average daily attendance over the six testing days was more than two standard deviations below the mean. These animals were filmed for assessment purposes on Day 7 after arrival at the feedlot.

6.3.2.2 Lame group

Lameness is recognised as a major negative welfare state in sheep (Lynch et al., 1992; Goddard et al., 2006). For 6 days after arrival at the feedlot, sheep suspected of being lame were identified and filmed. The video camera footage was viewed by two independent assessors to confirm the level of lameness. The assessors were experienced in evaluating sheep lameness and scored the sheep in the present study in accordance to a 0-6 lameness scale for sheep (Kaler and Green, 2008). Eight sheep were selected for the Lame group, with an average lameness score of 2.25 ± 0.32 (range 1.25-4.00), for filming and assessment on Day 7 after arrival at the feedlot.

6.3.2.3 Habituation group

Research has demonstrated that the habituation process can lead to animals displaying or exhibiting positive aspects of welfare state (Manteuffel et al., 2009). After arrival at the feedlot, a group of 51 sheep were randomly allocated to a separate pen from the rest of the sheep for the duration of the study. Daily, for 6 days, these sheep were calmly taken to an adjacent WoW setup and, as a group, gently moved by a stock person through the setup five times successively. On Day 7, eight individuals were randomly selected from this mob for filming and assessment, excluding sheep that could be classified as Lame or Inappetent, using the above criteria.

6.3.2.4 Control group

Following the allocation of the animals in the above three treatment groups, 12 sheep were randomly selected from the remaining pool for the Control group. This selection excluded any sheep that could be classified as Lame, Habituated, or Inappetent using the above criteria.

6.3.3 Walk-over-weigh (WoW) filming

On Day 7 after arrival at the feedlot, the 36 assessment sheep in the four treatment groups were individually filmed as they walked, of their own volition, through the WoW setup (Figure 6.1. The WoW setup was designed to simulate a common on-farm system where the sheep move from an open area through a narrow passageway (raceway or race), where the weigh scales are located, to gain access to feed or water on the other side. Weights (weight change) were not recorded in this experiment as there were no corresponding longitudinal measures of behaviour. Six additional randomly-selected sheep were placed in the open pen at the end of the simulated WoW setup, along with some hay, to provide motivation for the sheep to move through the setup without human intervention, and to avoid them returning back through the WoW (NB: this was not provided during the preceding 6-day process for the Habituated group, and only the Habituated group had previously encountered the WoW setup prior to filming on Day 7). All sheep to be filmed were held in a pen at the entrance to the WoW setup and were released individually, in random order, into the WoW setup. A human handler released each sheep from the holding pen by opening the gate after ensuring that the previous sheep had completely traversed the WoW setup. Time taken to traverse the WoW setup was recorded from the time that the sheep were released until they exited from the raceway (Figure 6.1). Flight speed was measured over a distance of 4 m immediately after exit from the holding pen, with markers at 1 m intervals along the boundary fence. The time taken for each sheep to travel the 4 m was calculated from the video footage collected from a camera positioned directly opposite the boundary fence at the exit of the holding pen (Camera 3; Figure 6.1). Specifically, speed (m/s) was calculated using frame-by-frame analysis of the video footage, given the known frame rate of the video camera (25 frames/s), to count the number of frames from when the sheep first exited the holding pen to when it first crossed the 4 m marker.

The collection of footage for behavioural assessment was achieved using two remotely operated digital video cameras (GoPro Hero3+: GoPro Inc., San Mateo, CA, USA) positioned to capture footage as the sheep approached the raceway, and as they travelled through the raceway (Cameras 1 & 2; Figure 6.1). Footage from these two cameras was combined (spliced together using the software package: Filmora for Windows, Wondershare, Vancouver, Canada) for each individual sheep to provide a continuous clip depicting the animals walking towards the race and through it, with an average clip length of 31 ± 18 s (mean ± s.d.).

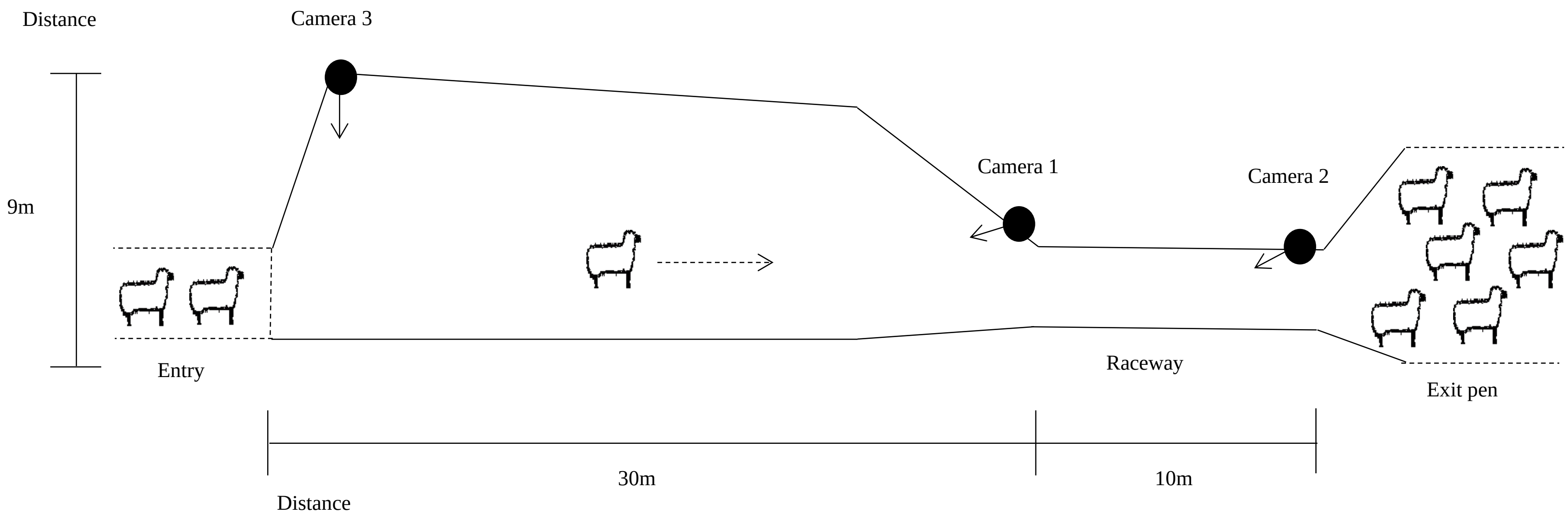

Figure 6.1. Schematic overview of the walk-over-weigh (WoW) setup. The dashed arrow indicates the direction of movement, and the camera locations and orientations are indicated by solid circles with arrows.

6.3.4 Behavioural measures

6.3.4.1 Quantitative measures

Behavioural aspects of physical health and/or the emotional state of the animals were examined using a range of quantitative measures, described in Table 6.1. The behavioural parameters selected have been investigated previously and interpretations of measures have been offered in terms of welfare state, i.e. positive, negative or neutral (Cockram, 2004; Greiveldinger et al., 2009; Llonch et al., 2015). A single observer, blind to experimental treatment groups, recorded the frequency and/or proportion of time the animals spent doing the various behaviours from the 36 individual video clips.

6.3.4.2 Qualitative behavioural assessment (QBA)

The combined video camera footage from Cameras 1 and 2, recorded as the 36 sheep traversed the WoW setup, was used for QBA. A total of 18 observers were recruited for this study from Murdoch University staff and students (14 female and 4 male). Observers were given detailed instructions on completing the QBA scoring sessions but were not given any details on the animals or the experimental treatments. Observers completed a short survey regarding their past experiences with sheep and other domestic livestock species prior to completion of the QBA assessment procedure. To complete the QBA by means of a Free Choice Profiling procedure, observers were required to attend two sessions; a term generation session followed by the quantification session.

Table 6.1. Description of the quantitative behavioural parameters used for the assessment of the sheep.

Behavioural measures	Description
Flight speed (*m/s*)	Speed of exit from holding pen, measured over 4 m.
Locomotor activity:	
Time taken to traverse the WoW setup (*s*)	Total time taken to traverse the Walk-over-weigh (WoW) setup, from the time the animal voluntarily moved into the set up from the holding pen until they exited the raceway.
Time spent in motion (*s*)	Proportion of time spent in motion, in any direction.
Time spent not in motion (s)	Proportion of time spent not in motion.
Circling incidences (*no.*)	Incidences of the animal turning around or changing direction greater than 90º.
Baulking (*no.*)	Incidences of stopping or circling within 1.5 m of the entrance of the race.
Vocalisations (*bleats/min*)	Incidences of an audible bleat.
Sniffing fixtures (*no.*)	Incidences of sniffing, nosing or rubbing at fixtures.
Total number of head movements (*no.*)	Total number of lateral head movements.
Percentage abnormal head posture at entrance to race (*%*)	Proportion of animals in each treatment group that exhibited low head carriage (head below withers) with or without an extended neck, as they entered the race section of the WoW setup.
Total number of ear movements (*no.*)	Total number of changes in ear posture (between asymmetrical, raised, back and passive postures).
Duration of ear positions:	Proportion of time with ears in the following positions:
Asymmetrical	Both ears in distinct positions with regard to the frontal plane, with corresponding asymmetrical visibility of auricles from the front.
Raised	Both ears in the same position, either in front of or in line with the frontal plane, with auricles visible from the front.
Back	Both ears in the same position, behind the frontal plane, with auricles concealed from the front.
Passive	Both ears in the same position, in the frontal plane, with auricles concealed from the front.

6.3.4.2.1 Term generation (Session 1)

Observers were shown 10 video clips, which were not used in the assessment session, depicting individual sheep traversing the WoW setup. Video clips were chosen that demonstrated a variety of behavioural expressions to allow observers to describe as many aspects of the sheep's expressive repertoire as possible. After watching each clip, observers were given 2 min to write down terms they thought described the animal's behavioural expression. There was no limit imposed on the number of descriptive terms an observer could generate, but terms needed to describe not what the animal was doing (i.e. physical descriptions of the animal such as vocalising, chewing, tail flicking), but how the animal behaved (e.g. *'nervous'*, *'relaxed'*). Subsequent editing of the descriptive terms was carried out to remove terms that described actions, and terms that were in the negative form were transformed to the positive for ease of scoring (e.g. *'unhappy'* became *'happy'*). The result being a unique list of descriptive terms for each of the 18 observers to be used for quantification in Session 2. Each descriptive term was attached to a visual analogue scale (minimum to maximum) in an electronic worksheet (Microsoft Excel 2003, North Ryde, NSW, Australia). The list of terms was randomly arranged, although terms with a similar meaning were not listed together.

6.3.4.2.2 Quantification (Session 2)

Observers viewed and scored video clips of the 36 assessment sheep using their own unique lists of descriptive terms. Observers were instructed to score each animal's expression using the visual analogue scale, where maximum indicated the animal could not show an expression more strongly and minimum reflected the absence of expression of that particular descriptive term and the distance between the minimum-point and their mark on the scale as reflected the intensity of each animal's expression on that term.

6.3.5 Statistical analysis

For QBA, the distance from the start of the visual analogue scale to where the observer had made a mark for each term was calculated (where minimum = 0 and maximum = 100) and these data were analysed by means of Generalised Procrustes Analysis (GPA) (Genstat 2008, VSN International, Hemel Hempsteat, UK; Wemelsfelder et al. (2000)). For a detailed description of GPA analysis and output interpretation procedures see Wemelsfelder and colleagues (2000; 2001). Briefly, GPA is a multivariate technique that identifies underlying patterns in observer assessments (i.e. descriptive terms of the animal's behavioural expression) and calculates the level of consensus between observer assessments of sheep. The statistical process whereby this best-fit pattern, termed the consensus profile, is identified takes place independently of the meaning of individual terms used by observers. The Procrustes statistic is calculated by quantifying the percentage of variation between observers (in their assessment of individual sheep) that is explained by the consensus. The statistical performance of the consensus profile above chance is calculated by comparing (using a one-sample *t*-test) the Procrustes statistic to the mean of a simulated distribution of 100 Procrustes statistics generated through 100 iterations of the analysis, where the data is randomised in a different permutation each time. Significance values in that test of $P < 0.001$ or better can be taken as evidence that the consensus profile was not a methodological artefact and does represent a common pattern identified by observers. The Procrustes statistic is also used to assess the degree of agreement between individual observers and the overall consensus profile. To do this, Principal Component Analysis is used to reduce the many dimensions within the consensus profile to a smaller number of dimensions, which explain the majority of variation between observed animals. To allow for semantic interpretation of these main dimensions, the score for individual observer terms can be correlated with the overall dimension score (i.e. the more highly correlated an individual term is with a dimension, the more weight it has as a descriptor – positive or negative – for that dimension). This process is entirely *post hoc* to the computation of the consensus profile but allows identification of the individual terms that best describe the anchor points at each end of the main dimensions for purposes of interpretation.

Treatment effects on the quantitative behavioural measures collected were analysed using Mann-Whitney *U*-tests. Chi-square analysis was used to test for the percentage of animals with abnormal head position upon entering the race (Genstat 2008, VSN International, UK). In addition, analysis of similarity (ANOSIM) and percentage similarity (SIMPER) analyses were performed by analysing data on all quantitative behavioural variables collected for the 36 sheep. The values were standardised and similarity was based on the Euclidean similarity measure. The sheep's scores on GPA dimension 1 were not normally distributed and could not be transformed adequately for parametric analysis. Treatment effects on these dimension scores were analysed using Kruskal-Wallis one-way ANOVA by Ranks with post-hoc Mann-Whitney *U*-tests (Genstat 2008, VSN International, UK). The association between the quantitative behavioural parameters collected and GPA scores (obtained from the QBA) for the 36 video clips were investigated using Spearman Rank Order correlation (Genstat 2008, VSN International, UK).

6.4 RESULTS

6.4.1 Quantitative behaviours

The flight speed of sheep traversing the WoW setup differed significantly between treatments ($P < 0.05$; Table 6.2). Specifically, sheep in the Habituated and Inappetent groups recorded 35% and 38% faster flight speeds, respectively, when compared to the Control animals ($U = 15$, $P < 0.05$ and $U = 20$, $P < 0.05$, respectively), whereas those in the Lame group had flight speed similar to that of the Control ($U = 37$, $P = 0.43$). Those animals in the Inappetent group also recorded fewer vocalisations than the Control ($U = 21$, $P < 0.05$; Table 6.2), whereas those in the Habituated and Lame groups recorded vocalisations similar to that of the Control ($U = 40.5$, $P = 0.59$ and $U = 42$, $P = 0.67$, respectively).The number of circling events also differed significantly between treatments ($P < 0.05$; Table 6.2), with animals in the Lame group recording fewer circling events than the Controls ($U = 20$, $P = 0.009$), but no difference recorded between the Habituated and Inappetent groups, and the Control group ($U = 29$, $P = 0.13$ and $U = 33.5$, $P = 0.26$, respectively).

There was no difference between the treatment groups with regard to the proportion of time the sheep spent in motion or not in motion, exploratory behaviour (sniffing), head movements, percentage of animals with abnormal head posture entering race, or incidences or proportional time of changes in ear positions (Table 6.2).

Table 6.2. Mean values of the quantitative behavioural parameters for the four experimental treatments (Control vs. Habituated vs. Lame vs. Inappetent) and correlation of values with qualitative behavioural assessment (QBA) scores.

Behavioural parameters	**Raw value**				**Spearman rank order correlation (R_s)**	
	Control	**Habituated**	**Lame**	**Inappetent**	**GPA dimension 1**	**GPA dimension 2**
Flight speed (*m/s*)	3.9±0.3*a*	5.3±0.4*b*	3.6±0.3*a*	5.4± 0.6*b*	0.04	0.33*
Traverse time (*s*)	30.6±5.1*a*	21.4±3.7*a*	29.0±6.4*a*	44.6±6.5*a*	-0.65***	-0.58***
Time spent in motion (*s*)	84.9±3.7*a*	91.7±4.9*a*	84.7±8.1*a*	76.1±4.0*a*	0.48**	0.50**
Time spent not in motion (*s*)	15.0±3.7*a*	8.3±4.9*a*	15.3±8.1*a*	23.8±4.0*a*	-0.48**	-0.50**
Circling incidences (*no.*)	1.0±0.4*a*	0.5±0.5*a*	0.0±0.0*b*	2.6±1.0*a*	-0.68***	-0.12
Baulking (*no.*)	1.0±0.35*a*	0.4±0.3*a*	0.4±0.3*a*	1.2±0.5*a*	-0.70***	-0.21
Vocalisations (*bleats/min*)	7.0±1.4*a*	6.0±1.7*a*	6.8±2.3*a*	3.6±2.0*b*	0.14	0.51**
Sniffing fixtures (*no.*)	0.0±0.0*a*	0.2±0.2*a*	0.4±0.3*a*	0.2±0.2*a*	0.28	-0.48**
Number of head movements (*no.*)	25.3±0.6*a*	23.1±0.8*a*	22.6±1.4*a*	27.5±0.9*a*	-0.27	-0.24
Percentage abnormal head posture at entrance to race (*%*)	58.3*a*	12.5*a*	12.5*a*	87.5*a*	-0.58***	0.03
Number of ear movements	10.3±1.9*a*	8.2±2.2*a*	12.0±4.1*a*	11.5±2.4*a*	-0.33	-0.49**
Asymmetrical (rel. duration)	0.15±0.04*a*	0.16±0.04*a*	0.13±0.04*a*	0.13±0.0*a*	0.02	-0.14
Raised (rel. duration)	0.71±0.09*a*	0.48±0.13*a*	0.75±0.08*a*	0.70±0.08*a*	-0.08	-0.19
Back (rel. duration)	0.06±0.04 *a*	0.03±0.02*a*	0.12±0.05*a*	0.17±0.05*a*	0.02	-0.14
Passive (rel. duration)	0.07±0.07*a*	0.33±0.16 *a*	0.00±0.00*a*	0.00± 0.00*a*	0.28	0.37*

Values are means ± S.E.M.. Ear position units (rel. duration) = relative duration. For raw values with significant treatment interactions, different letters indicate significant differences using Mann-Whitney U-tests (at $P < 0.05$), excluding percentage abnormal head posture where letters indicate significant differences using Chi-Squared (at $P < 0.05$). Correlations between behavioural parameters and GPA scores on dimensions 1 and 2 are presented, with significant effects indicated (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

ANOSIM revealed significant differences in the behaviour displayed by the sheep in the treatment groups in the WoW setup ($R = 0.11$, $P = 0.020$, average dissimilarity = 30.71). Specifically, ANOSIM identified that the differences in behaviours were greater between the Habituated and Inappetent groups ($R = 0.29$, $P = 0.029$, average dissimilarity = 36.19), and the Lame and Inappetent groups ($R = 0.26$, $P = 0.046$, average dissimilarity = 34.07), than within. The behaviours that contributed most to the variation seen between the treatment groups are displayed in Table 6.3. Briefly, proportion of time in passive ear position, sniffing fixtures, circling incidences, head position at entrance to race, time taken to traverse the WoW setup, and baulking contributed most of the variation seen between the Habituated and Inappetent groups. While flight speed, circling incidences, head position at entrance to race, number head movements, vocalisations, and time spent in motion contributed most to the variation seen between the Lame and Inappetent groups.

Table 6.3. Results of the similarity percentage (SIMPER) analysis of the quantitative parameters collected from the sheep in the four experimental treatments (Control v. Habituated v. Lame v. Inappetent). Parameters are ranked in order of contribution and only parameters that account for up to 50% cumulative contribution are shown.

Treatment	Overall average dissimilarity	Parameter (ranked in order of contribution)	Average dissimilarity	Contribution%	Cumulative%
Control v. Habituated	29.65	Passive ear position	3.888	13.11	13.11
		Raised ear position	2.926	9.866	22.98
		Sniffing fixtures	2.293	7.732	30.71
		No. ear movements	2.225	7.502	38.21
		Head position	2.215	7.469	45.68
		Flight speed	2.038	6.874	52.55
Control v. Lame	27.05	No. head movements	2.57	9.51	9.51
		Time spent in motion	2.45	9.07	18.58
		Time spent not in motion	2.45	9.06	27.64
		No. ear movements	2.21	8.18	35.82
		Asymmetrical ear position	2.09	7.71	43.53
		Back ear position	2.08	7.70	51.23
Control v. Inappetent	29.87	Flight speed	2.965	9.927	9.927
		Back ear position	2.698	9.032	18.96
		Baulking	2.68	8.972	27.93
		Traverse time	2.453	8.213	36.14
		Circling incidences	2.374	7.949	44.09
		No. ear movements	2.249	7.53	51.62
Habituated v. Lame	30.07	Passive ear position	3.67	12.20	12.20
		Sniffing fixtures	2.85	9.47	21.66
		Time spent in motion	2.72	9.03	30.69
		Time spent not in motion	2.71	9.02	39.72
		Raised ear position	2.70	8.99	48.70
		Flight speed	2.40	7.97	56.67
Habituated v. Inappetent	36.31	Passive ear position	3.67	10.10	10.10
		Sniffing fixtures	3.29	9.07	19.17
		Head position	3.19	8.79	27.96
		Circling incidences	3.15	8.68	36.64
		Traverse time	2.93	8.07	44.70
		Baulking	2.64	7.26	51.97
Lame v. Inappetent	34.13	Flight speed	3.37	9.87	9.87
		Head position	3.15	9.21	19.08
		Circling incidences	3.10	9.08	28.16
		No. head movements	2.75	8.06	36.21
		Vocalisations	2.63	7.70	43.92
		Time spent in motion	2.60	7.61	51.53

6.4.2 Qualitative behavioural assessment

The 18 observers participating in this study generated a total of 88 unique terms to describe the sheep they were shown (average 15 ± 1 terms per observer; range 8 - 25). The GPA consensus profile explained 58.2% of the variation between observer scores and this differed significantly from the mean randomised profile ($t99 = 80.65$, $P < 0.001$). The first two GPA dimensions identified within this consensus explained the majority (81.8%) of the variation in scores attributed to individual animals, with GPA dimension 1 explaining 73.1% and GPA dimension 2 explaining 8.7% (Figure 6.2). High scores on GPA dimension 1 were associated with the semantic correlation tags of *'focused'*, *'collected'* and *'assured'*, whereas low scores were associated with words such as *'reluctant'*, *'tense'* and *'wary'*. For GPA dimension 2, high scores were associated with terms such as *'rushed'*, *'energetic'* and *'aggressive'* and low scores with terms such as *'settled'*, *'thoughtful'* and *'quizzical'*.

Overall, there were significant treatment effects for the observer scores of the sheep on GPA dimension 1 (H_3, n = 15.44, $P < 0.001$), but not on GPA dimension 2 (H_3, n = 0.86, $P = 0.84$) (Figure 6.3). GPA dimension 1 scores for the Control group were significantly lower than both the Habituated ($U = 18.0$, $P < 0.05$) and Lame groups ($U = 15.0$, $P < 0.01$), with the observers rating the Lame and Habituated animals as more *'focused'*, *'collected'* and *'assured'*. The difference observed between the Inappetent and Control groups for GPA dimension 1, approached significance ($U = 25$, $P = 0.08$) with the observers tending to describe the Inappetent group as having a more *'reluctant'*, *'tense'* and *'wary'* demeanour.

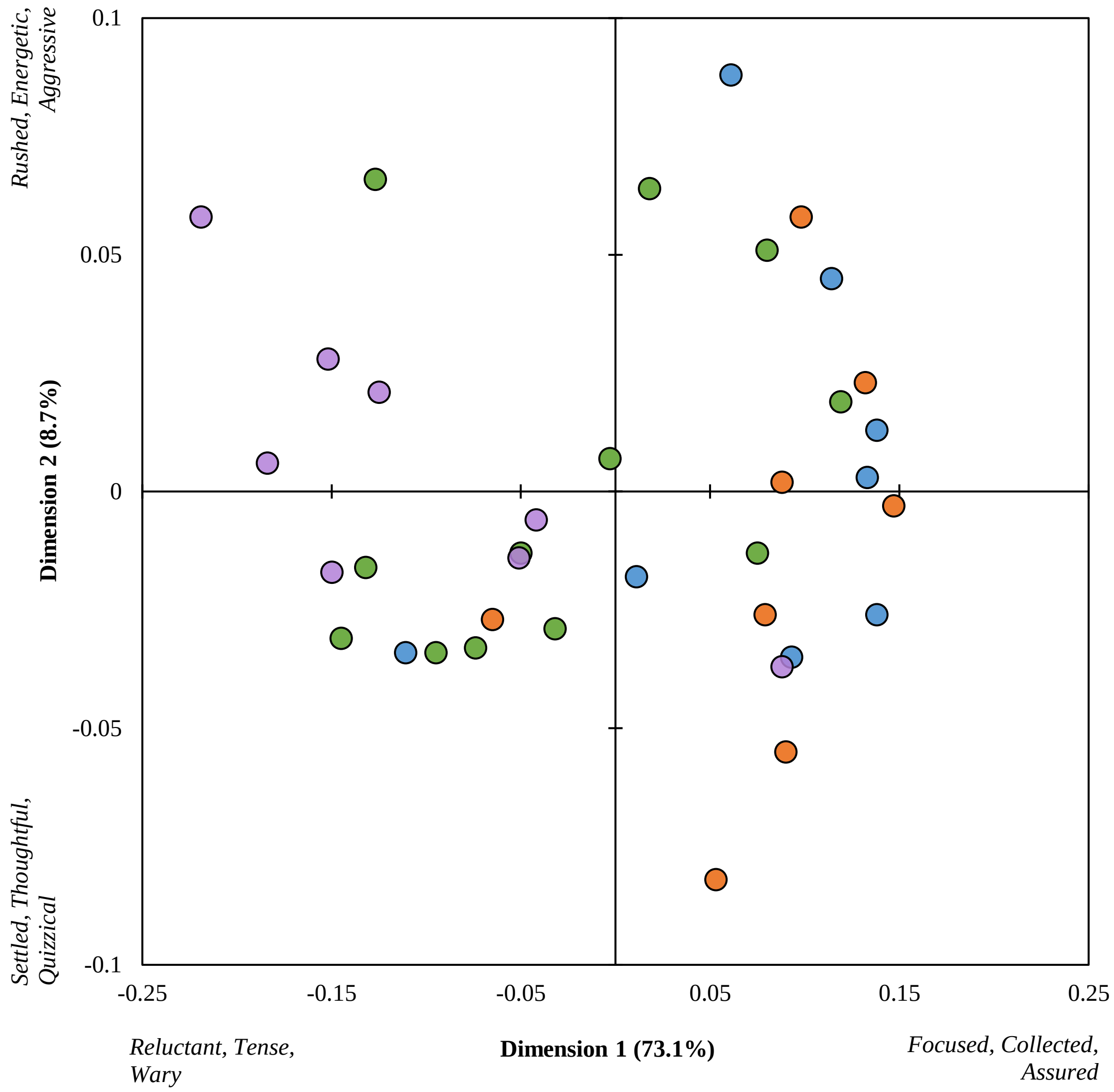

Figure 6.2. Positions of each individual sheep on the Generalised Procrustes Analysis (GPA) dimensions 1 and 2 resulting from qualitative behavioural assessment (QBA). Each animal is represented by a single data point; Control (green marker), Habituated (blue marker), Lame (orange marker) and Inappetent (purple marker).

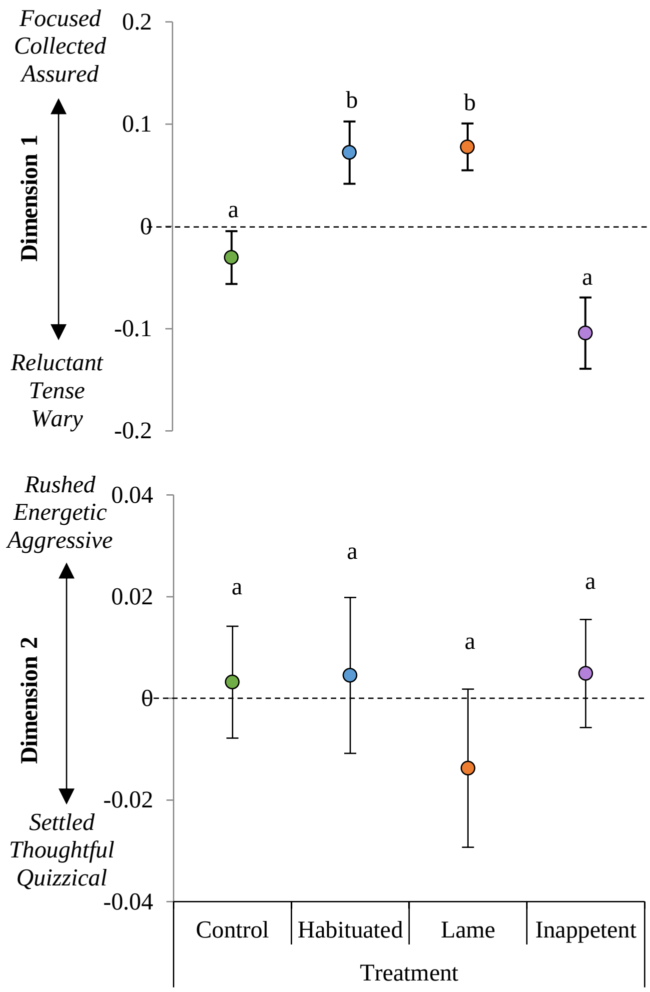

Figure 6.3. Means (± S.E.M.) of observer Generalised Procrustes Analysis (GPA) scores on (a) dimension 1, and (b) dimension 2, resulting from qualitative behavioural assessment (QBA) of sheep in four treatments; Control (green marker), Habituated (blue marker), Lame (orange marker) and Inappetent (purple marker). Within each dimension, different letters indicate treatment groups that were significantly different ($P < 0.05$).

6.4.3 Relationship between quantitative and qualitative measures of behaviour

A number of quantitative measures of behaviour recorded were significantly correlated with the GPA dimension scores (Table 6.2; Figure 6.4). Sheep described by the observers as being more *'focused/collected/assured'* on GPA dimension 1 (as opposed to more '*reluctant/tense/wary*') took a shorter time to traverse the WoW setup ($R_s = -0.65$; $P < 0.001$), spent a larger proportion of time walking ($R_s = 0.48$; $P < 0.01$), a smaller proportion of time standing ($R_s = -0.48$; $P < 0.01$), had fewer circling events ($R_s = -0.68$; $P < 0.001$), fewer baulking events ($R_s = -0.70$; $P < 0.001$), and had a normal head posture entering the race ($R_s = -0.58$; $P < 0.001$). Sheep described as more *'settled/thoughtful/quizzical'* on GPA dimension 2 (as opposed to more *'rushed/energetic/aggressive'*) had a lower initial flight speed ($R_s = 0.33$; $P < 0.05$), took longer to complete the WoW setup ($R_s = -0.58$; $P < 0.001$), spent a smaller proportion of time walking ($R_s = 0.50$; $P < 0.01$), a larger proportion of time standing ($R_s = -0.50$; $P < 0.01$), had fewer vocalisations ($R_s = 0.51$; $P < 0.01$), had more sniffing events ($R_s = -0.48$; $P < 0.01$), and a smaller proportion of time with their ears in the 'passive' orientation ($R_s = 0.37$; $P < 0.05$).

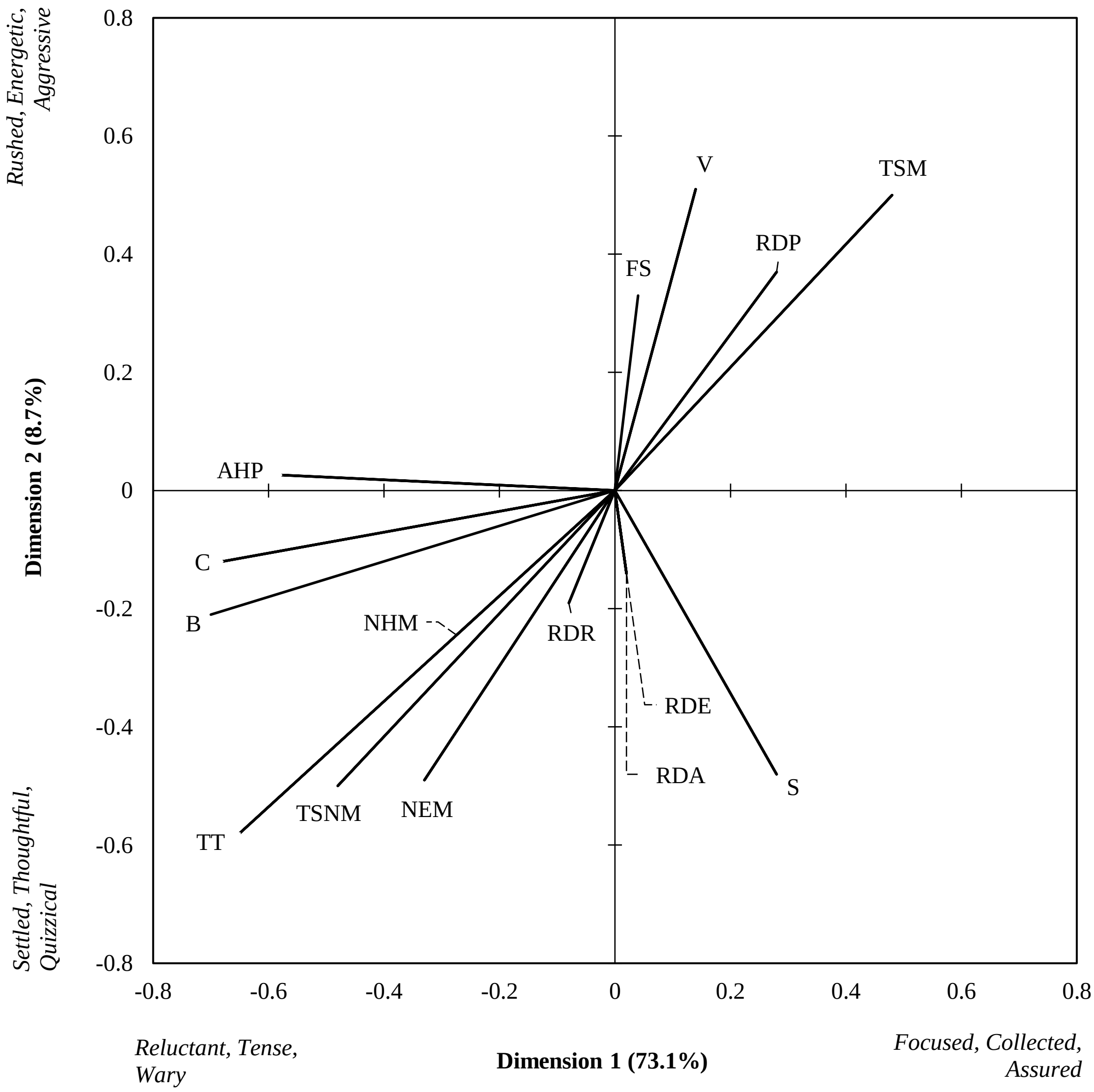

Figure 6.4. Spearman rank correlations between generalised Procrustes analysis (GPA) scores on dimensions 1 and 2, and the relative duration/frequency of the quantitative behaviour measures; Flight speed (FS); Traverse time (TT); Time spent in motion (TSM); Time spent not in motion (TSNM); Circling incidences (C); Baulking (B); Vocalisations (V); Sniffing fixtures (S); Number of head movements (NHM); Abnormal head position at entrance to race (AHP); Number of ear movements (NEM); and the relative duration spent in Asymmetrical (RDA); Raised (RDR); Back (RDB); and Passive (RDP) ear positions.

6.5 DISCUSSION

Sheep presumed to be in different welfare states showed different quantitative and qualitative behaviours analysed from short video clips automatically collected in a WoW setup. There was significant consensus between the observers in their assessment of the behavioural expression of the sheep in this study, and the QBA scores attributed to the sheep varied significantly between the groups investigated. Specifically, sheep that were categorised as Habituated or Lame displayed different behaviours from Control animals. These results are similar to previous studies in sheep (Wickham et al., 2012; Phythian et al., 2016) and indicate that the observers, who were blind to the experimental groups, could differentiate between the behavioural expression of sheep that were either injured (Lame), or acclimated to their immediate environment and human interaction (Habituated), and those that were not (Control) as they traversed a WoW setup. These findings support the hypothesis that using the QBA methodology, observers can provide scores that can add to quantitative behavioural measures to help distinguish sheep that are in different welfare states. These results suggest that there is potential, using QBA and key quantitative measures, for differences in particular aspects of sheep welfare to be identified remotely via autonomous data capture technology.

In the present study, both the Lame and Habituated groups were associated with the semantic correlation tags of *'focused/collected/assured'* when compared to Control animals in the GPA scoring system. This contrasts with results from a previous QBA study observing lameness in sheep where the observers described the Lame animals as having a *'distressed/dull/dejected'* demeanour (Phythian et al., 2016). To try to explain this difference it must be emphasised that in the QBA process, observers are not making any value judgements about the emotional or welfare state of Lame sheep, they are simply scoring the behavioural expression of a group of sheep using terminology that may be semantically-associated with emotions. In the present study, it is possible that the Lame sheep may have been experiencing pain or distress as a result of their lameness, but were attempting to minimise pain in their 'focused' locomotory behaviour. Indeed, the lower initial flight speed recorded for the Lame sheep compared to the Habituated sheep suggests that these animals may have been more careful as they entered the test arena. Moreover, the lower incidence of circling in the Lame animals compared to the Control sheep may reflect a choice of locomotion style that minimises pain and the risk of further injury. Others have suggested that sheep in pain will refrain from certain active movements or behaviours to reduce the intensity of pain (Molony et al., 1993; Molony and Kent, 1997). Perhaps the challenge of the WoW setup could be altered to allow for more differences in behavioural expression to become evident. For example, the inclusion of a simple locomotive challenge such as a step up. However, the inability to clearly distinguish the Lame animals from the Habituated in the present study has another important practical implication. It follows that after repeated exposure to the setup, as would occur in a practical situation, all animals exposed to the WoW setup may, eventually, become habituated. Therefore, to facilitate practical application, it would be important to investigate in future studies the impact of such habituation on the behaviour of animals in different welfare states within the WoW setup.

The observers did not identify differences in the behavioural expression between the sheep in a state of inanition and the Control animals using QBA. The behavioural expression of the sheep in the Inappetent group may have been too subtle for the observers within this study to identify. Sheep in a state of inanition often appear healthy, showing no obvious behavioural signs, making them difficult to diagnose before they die (House et al., 2006; Lightfoot, 2008). However, it is also possible the statistical power of the experimental design was not sufficient to differentiate the Inappetent sheep from the Controls using QBA. With a relatively small number of sheep, and the variation in degree of inappetence that these sheep would have had based on the selection protocol, it may not be surprising that the observers were unable to be differentiate them.

It appeared from the data that the qualitative assessment was somewhat driven by the locomotive measures. Sheep that had a faster flight speed (i.e. the Habituated and the Inappetent groups), were positively correlated with the observer scores of being more *‘rushed/energetic/aggressive’* on GPA dimension 2, and sheep that had a high number of circling incidences (Inappetent group), were positively correlated with the observer scores of being more reluctant/tense/wary on GPA dimension 1. The sheep in a state of inanition were perhaps more reactive to the stress resulting in the faster flight speeds. There is some evidence in the literature to suggest that temperament influences the ability of sheep to adapt to a stressor, with the animals that have a high reactivity in a temperament test having poorer performance traits, including feeding behaviours (Pajor et al., 2008; Dodd et al., 2012; Rice et al., 2016). Therefore, sheep in a state of inanition may have a temperament that makes them more reactive to the stress of a novel environment. For the Habituated sheep to also have faster flight speeds then seems counter-intuitive if it is assumed they would be less reactive to stress from being habituated to the WoW setup. Perhaps the faster flight speeds for this group reflect their eagerness to complete the task, for which they are accustomed, and join conspecifics. The circling behaviour observed in the present study might be explained by a conflict between the aversion to the upcoming narrow passageway of the WoW setup, and the motivation of a sheep to join its conspecifics (Lynch et al., 1992; p. 86).

It must be mentioned that the results of the multivariate analysis of the quantitative measures indicated that not only did the measures of locomotion contribute to the differences seen, but also postures and vigilance behaviours. Since sheep are likely to perform behaviours such as general increases in activity and vocalisations in a variety of circumstances, such responses can be difficult to interpret, particularly in terms of welfare (Rushen, 2000; Cockram, 2004; Gougoulis et al., 2010). This is why QBA has been proposed to help interpretation of results and thus to provide a clearer picture of the animal's state. QBA provides a ‘whole-animal’ assessment of body language that captures the subtle changes that may otherwise be missed when recording isolated behavioural events (Wemelsfelder, 1997; Wemelsfelder et al., 2001). It has been suggested that such assessments of body language can provide complementary information that is useful for interpretation purposes (Phythian et al., 2016).

6.6 CONCLUSIONS

In conclusion, sheep presumed to be in different welfare states were distinguishable using a combination of quantitative and qualitative behavioural measures analysed from short video clips automatically collected in a WoW setup. It appears that in using descriptive terminology to summarise details of behaviour, posture and movement, along with the context in which they occur, observers can use QBA to provide insight into the wellbeing of sheep, which may help investigators interpret behavioural scores in relation to presumed welfare state. Thus, these results suggest that the collection of behavioural data in a remote setup, like WoW, may be a beneficial and practical tool for sheep producers. This is the first study of its kind, and so has raised questions concerning the discriminatory power of such assessments and requirements of such an approach for practical application. However, the results point towards a promising future for automated biometric data capture technology, whereby the potential exists for practical behavioural assessment tools to be incorporated with such technology to improve farm animal welfare monitoring.

CHAPTER 7 GENERAL DISCUSSION

7.1 INTRODUCTION

The monitoring and assessment of welfare is important to the Australian sheep industry because good welfare improves production performance and society now expects high levels of care and humane treatment of production animals. Producers within the industry are committed to taking care of their animals, and consider welfare important (Doughty et al., 2017). Given the nature of sheep farming enterprises in Australia, welfare assessment tools should ideally be suited to extensive farming, such as pasture grazing, and be both cost-effective and easy to implement to maximise producer uptake and adoption. Such welfare tools in producer 'tool-kits' would facilitate animal monitoring, increase the skill of stockpersons to recognise relevant issues, and help identify those animals/flocks in need of attention. Under commercial conditions in Australia, behavioural assessments are best suited for this purpose and can provide insights into both the physical and psychological components of animal welfare. Assessing the welfare of sheep remains a considerable challenge in Australia, and few systematically evaluated behavioural assessments exist for this purpose. In order to address this and to ultimately improve the welfare of commercially-reared sheep, this thesis investigated both quantitative and qualitative methods to evaluate the behaviour of sheep subject to a number of welfare issues common for extensive systems.

The focus of this thesis was the application of the novel qualitative behavioural assessment (QBA) methodology to assess the behavioural expression of sheep with commercially-relevant welfare issues. There were two key reasons why the evaluation of the QBA methodology was the core of this thesis. One was that the approach is a promising tool to gain comprehensive insights into both the physical and psychological aspects of welfare that may guide the interpretation of other data (i.e. physical condition, physiological measures, and/or behavioural measures). The other reason is that the approach is suited for practical application, being quick, easy to implement and non-invasive. QBA has previously been incorporated into the various international animal welfare assessment protocols; Welfare Quality® and Animal Welfare Indicators Project (AWIN), and thus has the potential to be adopted commercially within Australia. There is considerable evidence that QBA is practical and the validity and reliability of the methodology have been well documented in numerous livestock species (reviewed by; Fleming et al., 2016), particularly pigs and cattle (e.g. Wemelsfelder et al., 2000; Rousing and Wemelsfelder, 2006; Stockman et al., 2011; Rutherford et al., 2012; de Boyer des Roches et al., 2018). However, the use of the QBA methodology to assess the behavioural expression of sheep has been less well studied, with only a few studies reported within the literature that address sheep welfare outside of transport conditions (e.g. Stockman et al., 2013b; Phythian et al., 2016; Serrapica et al., 2017), and there is the need to validate the approach in sheep under commercial conditions in Australia. The general aim of this thesis was to explore and evaluate the QBA methodology to assess the welfare of extensively reared sheep and to examine the relationship between quantitative and qualitative behaviours, as well as clinical observations, of animals that faced several common welfare issues that are relevant to the Australian sheep industry: flystrike, gastrointestinal parasitism, lameness, inappetence, and pain caused by husbandry procedures, as well as the perceived positive role of acclimation to human presence.

7.2 QUALITATIVE BEHAVIOURAL ASSESSMENT (QBA) AS A TOOL TO ASSESS SHEEP WELFARE AND IMPLICATIONS FOR THE AUSTRALIAN SHEEP INDUSTRY

Over four studies, this thesis demonstrated that, using the QBA methodology, observers that were blind to experimental procedures and treatments reached consensus in their interpretation of the behavioural expressions of sheep. Furthermore, these assessments related meaningfully to the welfare state of the assessed sheep as evidenced by significant associations with health/disease status, physical condition, and behavioural measures. Overall, the findings revealed that observers were able to distinguish the behavioural expression of sheep in varied welfare states using the QBA methodology.

Chapter 3 reports a study that compared the behaviour of flystruck and control (non-flystruck) sheep. Flystruck sheep were scored significantly differently on GPA dimension 1 (more *'exhausted/irritated'* compared with non-flystruck sheep), and this was correlated with the flystruck sheep spending less time grazing, more time standing abnormally and engaging in kicking, head turning, and biting their rump region. Another important finding of Chapter 3 was that the QBA behavioural expression scores given to each sheep corresponded to the severity of strike and the condition of the wool around the tail (breech). This finding was particularly encouraging given that the sheep in this study were only classified, based on the size of the flystruck area, as 'low' to 'moderately' struck. This chapter therefore represents the first confirmation that the QBA methodology may be used for the early identification of flystruck sheep which would not only improve welfare outcomes but minimise economic losses associated with the disease.

Chapter 4 reports the behavioural expression of sheep with differing levels of gastrointestinal parasitism over two experiments. In the first experiment, behavioural expression scores were related to the severity of parasitism as evidenced by the faecal egg counts (FEC) and mucous membrane anaemia scores. These results are promising from a welfare perspective as the ability to recognise when an animal's welfare state is deteriorating (e.g. sub-clinically parasitised) rather than when it is already extremely compromised (e.g. clinically parasitised), would enable producers to take corrective and/or preventative actions to help the animals before the condition progresses and worsens even further. In the second experiment, the anthelmintic treatment of parasitised sheep (subclinical and clinical) altered the behavioural expression scores of the focal sheep, suggesting that QBA could be used to monitor the progress of animals after treatment to detect 'improvements' in welfare state and identify any animals that may not be responding appropriately and are in need of further care/treatment. Previous studies seldom explore behaviour as a potential tool to identify parasitised animals and to inform welfare, with efforts focused on the development of clinical and physical measures such as FEC, dag scores and mucous membrane anaemia scoring for this purpose (e.g. Bath and van Wyk, 2009). Hence this chapter increases our understanding of the behaviour expressed by sheep when their welfare is challenged by gastrointestinal parasites.

Chapter 5 reports the findings of a study that investigated QBA as a tool to identify pain in sheep following routine husbandry procedures performed at lamb marking (ear tagging, castration, surgical mulesing, and tail docking). There were two key reasons why pain was chosen as the focus of this study. One was that commercially reared sheep in Australia are subject to numerous sporadic and routine practices that may cause pain. The other reason is that pain is arguably the most difficult state to recognise in sheep since they are stoic animals and have evolved to hide their pain from predators to avoid detection (Dwyer, 2004; Doyle, 2017). As such, the study was designed to test the sensitivity of QBA in recognising the perhaps subtle differences in the behavioural expression between individual lambs caused by pain. Findings from this study revealed that observers could perceive differences in behavioural expressions between control lambs and those that were subject to the painful husbandry procedures and were either given a placebo (saline) or analgesics (Tri-Solfen® and meloxicam) at 1.5 h post-procedure. However, we could not confirm whether the analgesic treatment administered normalised scores of behavioural expression as there was little evidence to suggest that the pain experienced by those animals who received it was lessened. Further work is needed to verify these results, and to evaluate the responses of lambs with and without 'effective' analgesic pain relief which is the gold-standard (see; Weary et al., 2006). Hence, even though no absolute conclusions can be drawn concerning whether QBA is a valid tool to identify pain in lambs, this study represents the first step in the validation process and reports some promising results for the application of the QBA methodology to identify and manage pain in sheep.

Finally, in an effort to evaluate the QBA methodology under conditions closer to those of true commercial systems, the study presented in Chapter 6 investigated multiple welfare issues at a pre-embarkment feedlot with the use of a test apparatus designed to simulate that of a walk-over-weighing (WoW) system that is routinely employed to weigh stock remotely. In doing so, we moved away from the more simplified and controlled conditions seen in the three preceding chapters and addressed not only the use of QBA to inform welfare when more than two treatments were presented, but importantly provide an example of how QBA may be applied under commercial conditions with the remote collection of video footage. The main finding from this study was that observers watching video footage, collected automatically within a WoW setup, were able to differentiate between sheep that were selected based on differing conditions of presumed welfare, both positive and negative. In this scenario, lameness and inappetence were considered negative (−ve) and habituation to the WoW setup and human interaction, positive (+ve), with control sheep also included in the study. Overall, this finding suggests that there is potential, using QBA, for differences in particular aspects of sheep welfare to be identified remotely via autonomous data capture technology. However, there remains aspects of sheep behaviour that were unable to be differentiated, for example, observers were unable to differentiate between the inappetent (−ve) and control sheep.

In and of itself, the application of QBA to assess the welfare of sheep from four different treatments (control v. habituated v. lame v. inappetent) was novel. Seldom do formal studies investigate more than two treatments due to the need to have greater power for statistical analysis, and these treatments are often directly contrasting (i.e. treatment v. control). However, the reality of commercial conditions means that it is unlikely that the sheep assessed will be from such clearly defined categories (i.e. healthy v. unhealthy), nor will there necessarily be only one welfare issue present that could affect the behaviour of those animals. As such, through the exploration of the behavioural expression of sheep from four treatments, Chapter 6 suggests a more realistic representation of the conditions that may be present in commercial systems.

For a comprehensive assessment of animal welfare, the identification of positive welfare is necessary. An important component of this thesis was the exploration of the behavioural expression of sheep that were in positive states. QBA was used to identify ‘improvements’ in sheep welfare state related to the treatment of parasitised sheep with an anthelminthic drench that markedly reduced parasite burdens (Chapter 34). In addition, observers used the QBA methodology to distinguish sheep that were habituated to humans and the experimental setup (Chapter 6), a process widely considered to have a positive effect on animals. The ability of observers to detect the positive effects of habituation in sheep, in particular habituation to transport, has previously been examined with promising results (Wickham et al., 2012). Together, these studies reveal that observers using the QBA methodology perceived the behavioural expression of sheep in presumably positive welfare states to be different from that of their conspecifics. Although the promotion of positive welfare states is a key aspect of welfare, the importance of the assessment and recognition of such states in sheep are often overlooked, and consequently few measures are available for this purpose. Thus, the research presented in this thesis not only offers insights into the behavioural expression of sheep subject to several relevant welfare issues that have not been previously reported, but the discovery that observers can reliably identify changes in behavioural expression related to positive welfare states expands the value of the QBA methodology as a potential on-farm monitoring tool. Hence, assessments of behavioural expression may offer a comprehensive assessment of welfare, useful in the assessment and recognition of both positive and negative aspects of welfare state.

This thesis has further validated the QBA methodology by relating behavioural expression scores to previously validated behavioural, clinical and/or physical measures of welfare throughout the various experimental chapters. Furthermore, this work has demonstrated that quantitative and qualitative behavioural measures complement each other, together providing a more comprehensive assessment to inform welfare. Without a ‘gold-standard’, against which the validity of QBA as a welfare measure can be tested, it is important that constant efforts are made to demonstrate that such assessments do indeed relate to the welfare of the animal. A key outcome of the work presented in this thesis is that several significant correlations with welfare measures were evident (Table 7.1). Collectively, observers identified and integrated differences in behavioural expression using QBA that were related to the welfare state of the sheep. QBA can therefore be used to meaningfully inform about sheep welfare and may help to both balance welfare assessments and make them more comprehensive.

Table 7.1. Summary of the 'main' semantic correlation tags, or descriptive terms, for each of the main GPA dimensions generated by the QBA analysis and their associations with those quantitative behavioural and physical conditions welfare measures collected in Chapters 3, 4 and 6.

Thesis chapter	GPA dimension	Semantic correlation tags (descriptive terms) of the main GPA dimensions	Association with other welfare measures collected
3.	1	Exhausted/irritated (compared to more positively occupied/assured)	↓ grazing ↑ abnormal standing ↑ restlessness ↑ kicking ↑ head turning ↑ biting rump region ↑ size of struck area ↑ dag score
	2	Inquisitive/collected (compared to more indecisive/depressed)	↑ walking ↑ tail wagging ↑ kicking ↑ head turning ↑ biting rump region
4. (Experiment 1)	1	Docile/at ease (compared to irritated/responsive)	↑ FEC*
	2	Assertive/motivated (compared to lazy/social)	↑ FEC*
6.	1	Reluctant/tense (compared to more focused/collected)	↓ walking ↓ normal head posture ↑ time to complete task ↑ standing ↑ circling incidences ↑ baulking
	2	Settled/thoughtful (compared to more rushed/energetic)	↓ flight speeds ↓ walking ↓ vocalisations ↓ passive ear posture ↑ time to complete task ↑ standing ↑ sniffing

*FEC; Faecal egg count.

This thesis demonstrates that QBA is a valuable tool that helps producers gain insight into the welfare state of their animals in situations where more conventional quantitative measures could not be easily collected. Indeed, while providing valuable information, most quantitative behavioural measures were developed for scientific purposes and are not practical or suitable for on-farm application being either laborious (e.g. time budgets) or difficult to assess accurately (e.g. ear posture). As demonstrated throughout this thesis, and the literature (e.g. Wickham et al., 2015; Phythian et al., 2016; de Boyer des Roches et al., 2018; Vindevoghel et al., 2019), QBA provides valuable information relevant to an animal's welfare state, information that when used in conjunction with other measures may facilitate the easier recognition of animals whose welfare states are either improving or deteriorating without requiring assessors to spend extended amounts of time capturing multiple measures of animal behaviour. Under commercial conditions, QBA could be used as a screening tool alongside some other “iceberg indicators” such as BCS and locomotive scoring to identify individuals whose welfare might be compromised and require a closer inspection or to compare animals over time for monitoring purposes.

The focus of this thesis was to test the QBA methodology as a measure in sheep to ultimately improve the welfare of sheep reared commercially in Australia. Hence, it is important to consider here how QBA may be applied formally to achieve this. Broadly, QBA may be applied in one of three key ways to improve welfare, i) adopted by producers as a tool to monitor stock either remotely, or strategically at key production times, ii) used by external parties (e.g. auditors) which could involve the submission of remotely collected video footage for review and feedback, or iii) as a training tool to improve stock person skills (e.g. recognition of the signs of illness or injury). Since the welfare of an animal under commercial conditions is ultimately determined by the decisions producers make (i.e. whether to monitor, inspect or treat animals), the improved ability of producers to monitor and detect those in compromised states would better the welfare of the animals under their care. The idea that QBA can be applied formally to assess sheep under commercial conditions to improve welfare has already been raised. Indeed, QBA is incorporated in the AWIN assessment protocol in sheep (AWIN, 2015d), and Phythian et al. (2016) has also demonstrated the usefulness of QBA to assess sheep on commercial farms. It is important to note here that these studies use the Fixed List approach to QBA, which is more suited for practical application than the FCP approach evaluated within this thesis. That the behavioural expression of sheep was not well studied or understood, and the FCP approach is reasoned to be more sensitive, were the key reasons for this choice. The freedom of observers to create numerous terms with various meanings allows for the capture of subtle behavioural difference, an important consideration when assessing a stoic species such as sheep. The use of FCP also solves issues surrounding the training of observers in the definitions of FL terms, a known area of concern (e.g. Minero et al., 2016; Muri and Stubsjøen, 2017; Arena et al., 2019). Moreover, it is common for the terms created in FCP studies to be the foundation of proposed terms in FL. For example, in the development of a FL for dogs, the highest loading terms from a previous FCP study were considered as the starting point for the development of the list, on which a review of the literature and expert consultation built (Arena et al., 2019). The studies in this thesis improve the understanding of the behavioural expression of sheep and provide a good foundation for future studies in which the sensitivity and validity of the FL approach could be tested.

Within this thesis, Chapter 6 provides a strong example for how assessments could be incorporated in a remote capture system, whereas the other three experimental chapters have demonstrated that robust and meaningful assessments of sheep behavioural expression can be made from video footage as short as 30 seconds. Although assessments in sheep have been largely performed from video footage, they can also utilise sheep that are observed directly (i.e. ‘live’) as demonstrated by Muri and Stubsjøen (2017) and Phythian et al. (2016). The value of such assessments is that stock persons could complete them as part of routine surveys, or at key production dates, to capture a picture of welfare over time. It is important to note that lower inter-observer reliability has been found when assessments were done ‘live’ when compared to that from video footage (e.g. Muri and Stubsjøen, 2017), which is a challenge that needs to be addressed. As such, the training of those persons that use QBA (e.g. stockpersons) must be addressed to reduce this source of bias to ultimately improve validity and facilitate future adoption. Overall, the work presented in this thesis together with that in the literature, demonstrates how versatile the QBA methodology can be, and how it may be adapted to suit the needs of those using it. For example, it may be tailored towards welfare audits, used by producers to survey stock to identify those in need of closer inspection, or be applied to evaluate different management practices (e.g. different husbandry systems, housing, or procedures). Thus, QBA shows promise for development into a useful welfare measure that sheep producers can add to their toolbox to support monitoring and assessment efforts and to ultimately improve the welfare of their stock.

7.3 LIMITATIONS, FUTURE STUDIES, AND FINAL THOUGHTS

There are some areas of experimental design that could be improved if given the opportunity and resources. Even though we demonstrated that behavioural expression scores altered with increasing parasite burden in Chapter 4, we did not have a naturally ‘unparasitised’ group of sheep, with only 2 of the 53 individuals sampled categorised with a FEC less than 50 epg. Therefore, comparisons between the behavioural expression of unparasitised, subclinical- and clinically-parasitised sheep could not be made. To do this, it would be necessary to incorporate a group of experimentally ‘unparasitised’ sheep, with the administration of an effective anthelmintic drench and confirmation of status for these animals prior to behavioural assessments. In addition, to validate the use of QBA to assess pain in lambs, it would be necessary to observe focal lambs further from the time of analgesic administration than performed in Chapter 5. This would ensure that the onset of action of Tri-Solfen® and meloxicam was reached so that a comparison between the behavioural expression of lambs that were and were not experiencing pain caused by the routine husbandry procedures (ear tagging, castration, surgical mulesing, and tail docking) could be made. It would be worthwhile to also investigate those painful husbandry procedures in isolation to form a more comprehensive picture of the nuances of the complex phenomena that is pain in sheep. From a broader view, the comparison between the assessment of the same animals ‘live’ compared to collected video footage would have been advantageous in these experiments. However, several logistical and management limitations (e.g., animal ethics, coordination of volunteers on commercial premises), and the potential confounding effect of a large number humans (18 – 35 volunteers) on the behaviour of focal sheep, meant that it was not feasible.

Importantly, to confirm responses in sheep behavioural expression reported in this thesis and to increase the robustness of the findings, it would be worthwhile to evaluate a larger number of animals in each instance. Larger sample sizes were not possible in this thesis either because of the difficulty in capturing suitable footage or since only a small number of animals were identified as sick or injured upon inspection. It became apparent during the filming of sheep for these studies that the collection of clear and useable video footage under the conditions tested was a challenge. In particular, the collection of suitable video footage of the focal animals in Chapters 3 – 5, which involved the recording of focal animals not only from a distance, but when surrounded by conspecifics that could, and did, completely obstruct the view of the focal animal was difficult. That we purposely blinded observers to the visual evidence of scouring (diarrhoea) and painful husbandry procedures (wounds, blood, rubber ring) on the focal animals also reduced the number of sheep with suitable footage for QBA in Chapters 4 and 5, respectively. It also became evident that since our studies were observational in nature; that we assessed the behavioural assessment of sheep that were naturally diseased, injured, and healthy, rather than experimentally manipulating or imposing their treatments (except for the habituated sheep in Chapter 6, and the anthelmintic treatment of all animals in Chapter 4), also reduced the number of animals available that met the criteria for filming and subsequent assessment in each chapter. For example, only eight sheep were identified as inappetent from the 877 sheep whose trough feeding behaviours were monitored and evaluated in Chapter 6. While it was perhaps possible to impose the desired treatments (e.g. treatment of sheep in Chapter 4 with a specific daily dose of a particular combination of gastrointestinal parasites), which would have improved the certainty of the results herein, we can learn much from the observation of sheep whose conditions were naturally-occurring under less constrained contexts. Not only does the assessment of sheep with naturally occurring poor welfare mean that experiments were less invasive, and the animals were disrupted less, but the results are also arguably more relevant to the industry as the responses of the sheep are likely to be more representative of those seen under commercial conditions.

It is also important to consider whether the length of observation influences the ability of observers to make accurate assessments of sheep behavioural expression. Due to the difficulty in capturing clear and uninterrupted video footage of individual focal animals, clips presented to observers for QBA in this thesis were of shorter duration than intended. Short observation times may not allow for a complete picture of each animal's behavioural expression to manifest or may perhaps lessen the ability of observers to notice and integrate the complex details of behavioural expression. The effect of the length of observation on QBA assessments has not yet been examined and is an obvious area for future study.

Challenges surrounding the collection of suitable footage may be circumvented by the assessment of a group of animals rather than the individual. Exploration of the behavioural expression of groups of sheep in different welfare states would be valuable considering that the assessment of a group of animals rather than the individual is commonplace in practical situations (Marchant-Forde, 2015). Importantly, it is arguably easier to use the QBA methodology on individual animals than a group of animals, as this allows for detailed and better observation of the postures, facial expressions, and activities of each animal. Although it is also recognised that this would not be feasible under commercial conditions due to inherent management restrictions (labour, time, and resources limitations). Regardless of whether assessments are done on an individual or a group of animals it is also relevant to determine whether footage depicting different views of the same animal/s influences the behavioural expression scores given by observers using the QBA methodology.

This thesis largely focused on the use of QBA to inform on sheep welfare although traditional quantitative behavioural measures that may prove useful to inform on sheep welfare under commercial conditions were also included. The use of return order and speed of return to identify sheep with high FEC in Chapter 4 is perhaps the most notable, although restlessness scoring and the recording of flight speed in Chapters 3 and 6, respectively, could also be promising tools for producers, particularly if these could be captured remotely. It is difficult to single out any specific behaviour that can be captured on-farm to meaningfully inform about the welfare of sheep that is also easy to collect. As the thesis progressed, it became apparent that some quantitative measures were less suited for collection under commercial conditions than others, being impractical or difficult to collect. For example, the incorporation of an assessment of ear posture, a novel yet valuable measure that has been proposed to provide useful information concerning both positive and negative welfare in sheep (e.g. Reefmann et al., 2009c; Boissy et al., 2011), was collected in Chapter 6 but could not be reliably or accurately collected in the other studies that contribute to this thesis. The investigation of the validity and feasibility of such quantitative behavioural measures to inform sheep welfare is beyond the scope of this thesis, but the results presented herein justify future investigations.

Broadly, there are many directions future research into the use of the QBA methodology to assess sheep welfare could take. First, studies are required to refine the methodology to create a useful and valid tool for producers which could include:

(1) Determination of the ideal length of observation required to make accurate and reliable assessments of sheep behavioural expression.
(2) Evaluation of the use of video footage for QBA, compared with 'live' pen-side assessments.
(3) Improved understanding of how human presence influences such assessments.
(4) The evaluation of the use of predetermined lists of descriptive terms (i.e. fixed lists; FL) such as that provided in the AWIN protocol for sheep (AWIN, 2015d). In addition, it would be valuable to investigate the sensitivity of such FLs to score sheep behavioural expression, compared with the FCP approach, as done by Clarke et al. (2016) in pigs.

The determination of these three matters would greatly advance the development of the QBA methodology for practical application in sheep.

Second, future studies could expand the range of welfare issues or states in which the QBA has been evaluated in sheep. For example, while we examined the behavioural expression of sheep subject to several of the common welfare issues relevant to the Australian sheep industry, the evaluation of sheep subject to other important issues such as pregnancy toxaemia, undernutrition, and stress caused by shearing, weaning, or poor human-animal interactions, would be worthwhile. In particular, additional work to study the behavioural expression of sheep in positive welfare states is necessary. It would also be beneficial for further studies to address those conditions considered herein in more detail. For instance, investigating different types of pain (e.g. ischemic and inflammatory pain, visceral and somatic or superficial pain, and neuropathic pain; and acute vs. chronic pain) and pain of different severity than considered in Chapter 5, or infection with relevant gastrointestinal parasites (e.g. black scour worms; *Trichostrongylus* spp.) at a clinical level rather than subclinical as considered in Chapter 4.

Third, whilst we focused on validating QBA against other behavioural data, there are numerous other measures (e.g. physical health/condition and physiological measures) available that could be included in future studies to further validate the methodology in sheep. Moreover, since we were restricted in those quantitative behaviours we could collect herein, there is value in incorporating more extensive quantitative behavioural assessments in future studies. Perhaps the use of biosensors such as accelerometers would facilitate such assessments as proposed in Chapter 4. Likewise, the adoption of an automated system to detect ear posture and/or facial expression as done by McLennan and Mahmoud (2019) may also prove valuable in this regard. Such studies would not only contribute to ongoing validation efforts but would improve our understanding of the responses of sheep under these conditions.

Finally, as our recruitment efforts yielded predominantly inexperienced observers, it would be worthwhile to focus recruitment to observers that are experts or have experience in sheep and their behaviour, such as stock persons, particularly since it is such personnel that may ultimately use the approach. It would also be useful to conduct a survey to consult with such personnel, which may potentially highlight aspects of the methodology or barriers to adoption that need to be addressed which have not yet been considered, and to ultimately determine if they are likely to adopt the methodology.

Collectively, the studies presented within this thesis demonstrate that observers can use the QBA methodology to detect differences in the behavioural expression of sheep in different states of welfare. It demonstrates that QBA offers both relevant and valid assessments of behavioural expression that may help producers gain an insight into the welfare state of their sheep. Furthermore, each experimental chapter presented herein is novel and valuable, contributing a great deal to the understanding of the behavioural expression of sheep under these conditions which have never before been investigated. The work presented in this thesis has also drawn attention to many new questions and has highlighted issues that need to be addressed to further the development of the methodology for practical use in sheep. The research detailed in this thesis provides a solid foundation for the future studies of this kind that are needed to better our understanding of the behavioural expression of sheep in both good and poor welfare states and to further validate and refine the QBA methodology. Overall, the results point to a promising future in which QBA could be a valuable tool for producers to ultimately improve the welfare of sheep in their care.

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