

Body temperature as a remote measure of health in sheep

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Abstract

The measurement of body temperature in sheep has been a well investigated topic, providing insights into the health and physiological state of animals. Many common illnesses and disease are associated with a change in body temperature. The ability to extend this measurement into extensive production systems would be of benefit to producers, researchers and anyone interested in the health of their animals. The aim of this thesis was to identify common health issues and indicators, explore one of the most suitable indicators in terms of its applicability to animal health measurement, and investigate the available technologies which may be of use in remote monitoring of sheep health.

The Chapter 2 literature review section of this thesis has presented an overview of the current knowledge regarding health measurement in sheep, focusing on temperature measurement as a useful indicator of common health issues experienced by sheep in extensive production systems. It then explores the currently available technologies with which remote temperature measurement may be possible.

Chapter 3 aimed to investigate how well body temperature measured at peripheral sites in the neck muscle (T_n) and tail subcutaneous measure (T_t) reflected core (vaginal) temperature (T_v) detection and the daily rhythmicity in the body temperature of sheep. The impact of feeding frequency on both core and peripheral temperature measures was also investigated. While absolute and fitted temperature parameters differed between T_v, T_n and T_t, pattern similarities were observed at time points across the measurement day, with T_v, T_n and T_t exhibiting similar rhythmicity per the time of day. Additionally, feeding schedule may impact on short term fluctuations over the day in body temperature, but not on overall rhythmicity and temperature range.

Chapter 4 aimed to investigate the similarities between core and two peripheral sites when measuring temperature changes of sheep under a fever challenge. Significant differences were observed between challenge and baseline cosinor adjusted data for all parameters for T_v, and most for T_n while, for the purposes of fever detection or detection of clinically significant temperature change, subcutaneous T_t measurements showed minimal detection of treatment effect ($P < 0.05$).

In Chapter 5 an initial investigation and then a main experiment were conducted to investigate the potential for infrared thermography (IRT) to detect skin inflammation in sheep, particularly with regards to inflammation associated with fly strike. In the main experiment,

significant differences were found between control and sites injected with vaccine formulation (Covexin©10) for most of the experimental period for the short wool length, while fewer significant differences were found between injection and control sites for medium wool and long wool lengths ($P < 0.05$). Variation was lower across the measurements for short wool compared to medium and long wool. These findings suggest that IRT may have application in the detection of skin inflammation in sheep at short wool lengths, and may have limited application at longer wool lengths.

The experiments undertaken as part of this thesis have identified and demonstrated the capabilities and limitations of temperature sensing microchips and infrared thermography and informed as to their possible applications for remote temperature monitoring in sheep.

Declaration

I hereby declare that this thesis is an original work produced as part of a Master of Rural Science and has not been submitted for any other degree or qualification.

I certify that any assistance received in preparing this thesis and all sources used have been acknowledged.



Tellisa R. Kearton

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List of Abbreviations

CIDR	Controlled intravaginal drug release
DM	Dry matter
FS	Feeding schedule
IRT	Infrared thermography
LPS	Lipopolysaccharide
ME	Metabolisable energy
NCIT	Non-contact infrared thermometer
OJD	Ovine Johnes disease
RFID	Radio-frequency identification
SD	Standard deviation
SE	Standard error
Ta	Temperature measurement ambient
Tn	Temperature measurement neck
Tt	Temperature measurement tail
Tv	Temperature measurement vagina

Chapter 1

General introduction

1.1 Introduction

Research into remote animal health monitoring is developing as we enter a new technological age, with sensors and devices becoming more portable, affordable and versatile across a range of applications (Banhazi and Black 2009). As a result, the development of new remote monitoring techniques using emerging technologies requires careful investigation to ensure maximum benefits can be achieved in improving the health and wellbeing of extensively raised animals.

Monitoring sheep presents unique challenges, both in terms of their physiology and also in terms of their behaviour, and susceptibility to stress through handling (Hargreaves and Hutson 1990b). This encourages investigators to deliver producers and researchers with techniques and equipment which reduces handling and improves health monitoring while enjoying the health and production benefits provided by extensive production systems. It also requires investigation into the thermophysiological factors which impact on measurement devices and locations, in addition to understanding characteristics unique to sheep, such as the impact of wool on these measures (Lyne and Hollis 1968).

Body temperature is an important health and diagnostic indicator. Variation in body temperature can occur due to individual differences, circadian rhythmicity, and challenge states in which the animal may be unable to maintain thermal integrity, or in which a thermal state is produced as a result of a response to infection or injury. Understanding rhythmicity of body temperature assists in the development of effective strategies in the detection of illness and disease, and the role that different regions of the body play in the maintenance of thermal homeostasis (Refinetti 1992).

This thesis aims to describe the mechanisms which influence body temperature measurement, investigate the potential of two devices for temperature measurement in terms of their application in disease and illness detection in sheep, and develop an understanding of the physiological effects on temperature measurement and analysis. It will examine the available technologies, and focus on two of these devices, investigating their potential application in body temperature measurement, detection of abnormal temperature characteristics, and detection of skin inflammation.

The following literature review outlines the current state of research both into remote monitoring technologies, and also the physiological challenges which must be considered when investigating methods of remote monitoring as they relate to body temperature.

Chapter 2

Literature Review

2.1: Context

Animal health and wellbeing is an increasing concern for both consumers and producers alike. Extensive production systems present unique challenges in identifying and treating common illnesses and health issues in sheep, and it is difficult to monitor the health and wellbeing of extensively raised sheep (Morgan-Davies *et al.* 2006), particularly in Australia. An economic report compiled by Lane *et al.* (2015) for Meat and Livestock Australia (MLA) estimated the annual costs of a number of common illnesses of sheep, with perinatal mortalities heading the list costing \$540M nationally, followed by internal parasites at \$435M, dystocia at \$219M, weaner ill thrift at \$187M and flystrike at \$173M. Other common illnesses of sheep of economic significance detailed in the MLA report include bacterial enteritis, clostridial disease, footrot and ovine Johne's disease (OJD) and many others, with combined losses attributable to health issues in sheep totally over two billion AUD (Lane *et al.* 2015).

In the context of illness and disease, the ability or otherwise of an animal to maintain its internal environment in a state of homeostasis, or more recently allostasis (Korte *et al.* 2007; Linklater and Gedir 2011) is an indicator of the health status of an animal. Additionally, the physiological responses to challenges, such as the fever response, can alert animal handlers to the onset of disease or illness. Some of the contributors to health issues in extensive systems include predation (Manning *et al.* 2014), disease and illness. Fly strike, for example, results in a stress and inflammatory response due to the pain and irritation caused by blowfly larvae (Seaton *et al.* 1992). These causes of poor health impact on productivity (Hemsworth *et al.* 2000), and cause pain, discomfort and possibly death (Fitzpatrick *et al.* 2006). Improved health through diagnosis and early treatment of illness and disease is of benefit to livestock managers, as less product is lost to illness and disease, supply is maintained, and animal health priorities are addressed more efficiently. Good animal health management practices include the prompt detection and treatment of illness and disease, and this is of particular importance in extensive systems where lack of continual monitoring may result in delays in detection of ill or injured sheep (Dwyer 2009). Currently there are few resources for monitoring the health of livestock in extensive systems and these animals may face different health challenges than their more intensively managed counterparts. Therefore a more comprehensive monitoring method is needed for these production systems. Development of a remote and automated monitoring system to improve animal health outcomes in extensive systems is an identified goal for a number of animal industries in Australia, in which extensive production still comprises a large proportion of animal production industries.

The following literature review outlines the current knowledge surrounding remote health and wellbeing measurement, particularly in sheep raised in extensive environments, and seeks to identify the gaps in the knowledge. The potential for use of modern technologies and devices will also be examined with a view to testing the most promising of these technologies as part of the development of a remote health and wellbeing system.

2.2 Traditional indicators of health of sheep

2.2.1 Behaviour

There are a number of traditional measures of health that have been used in animal production. Some of the most commonly utilised measures are behavioural indicators, such as depression, lack of appetite, avoidance behaviour (Caroprese 2008) and lethargy (Barnett and Hemsworth 1990). Behaviour can be a useful indicator of disease because while many prey animals do not show discomfort, some diseases may invoke certain behaviours, which are recognizable. For example, lethargy, depression, anorexia and a reduction in grooming behaviour are commonly observed in association with febrile infectious disease (Hart 1988), while Broom (2006) describes kneeling behaviour of sheep suffering foot rot as an aid in diagnosis. Work is being undertaken in the field of automated monitoring of behaviours using equipment such as video (Sneddon and Mason 2014) and audio (Galli *et al.* 2011) monitors, and these may go some way to addressing the difficulties in extensive sheep health monitoring.

When looking at the behaviour and health of extensively farmed animals, an aspect of health which is intrinsically perceived as “good” is that extensively farmed animals have more freedom to express and control their behaviour (Deag 1996). The expression of normal behaviour is described as one of the “Five Freedoms” which were developed by the Farm Animal Welfare Council (Brambell 1965) to provide a framework through which the aim of good health and consequently animal wellbeing could be achieved (McCulloch 2012). As a result of the limitations imposed by the nature of extensive production it is important to identify measures of sheep health which can provide a picture of the overall health and wellbeing of animals in an extensive environment. Through the monitoring of behavioural characteristics and activity levels, there is potential to use these parameters in a diagnostic capacity for detection of illness and disease (Broom 2006; McLennan *et al.* 2015). While many measures of behaviour have their origins in subjective observations, research and development of these measurements have identified strong associations between the observed behavior and other more objective measures of health and wellbeing. Additionally, development of methodologies such as the Quantitative Behavioural Assessment (QBA) which aim to develop a “whole-animal” methodology in the interpretation of behaviour, show promise in the advancement of animal health monitoring and illness detection (Stockman *et al.* 2011). Although observations of behavior are a more subjective measure, they can be useful in compiling a “health profile” for individual animals, allowing for a more comprehensive picture for the assessment of sheep health.

2.2.2 Body weight and scoring

Other measures of health and wellbeing in sheep, such as body condition scoring (BCS), are usually limited to longer term, chronic conditions and do not indicate early onset of illness or disease (Caroprese 2008). Body weight can be an indicator of stress and or illness. Experimentally fly struck sheep showed a significant liveweight change when compared to control animals within the first week of infection of a 42 day trial period (Colditz *et al.* 2005). Body weight measurement in sheep is currently being investigated as an option for use as part of an integrated automated remote monitoring system with technology such as a walk-over-weighing system (Morris *et al.* 2012). As a result, body condition and liveweight is a useful measure, albeit with a lag or delay in the case of illness and disease detection, of sheep health and provides an important objective measurement to assist as part of a more comprehensive monitoring system (Morris *et al.* 2012).

2.2.3 Husbandry practices

Husbandry is an important factor in the assessment of sheep health and wellbeing, the ability of animal carers to identify and treat ill animals varies greatly and requires not only extensive training but also can be time consuming. Sheep in extensive environments are less able to be observed by experienced stockpersons on a regular and ongoing basis, therefore illness and disease may be well progressed by the time an experienced handler is able to view sheep (Dwyer 2008). Stockmanship has been identified as a critical factor in the management of intensively raised animals, both in the improvement of health and wellbeing (Hemsworth *et al.* 2009) and in productivity (Hemsworth *et al.* 2000). Therefore, any health measurement system which involves handling should also take this important aspect into account. Extensive sheep production systems are common in Australia, with sheep predominantly grazed rather than fed, except during times of food shortages and supplementation. Extensive systems have the perceived advantage to the animals of freedom to express natural behaviours, and as such are viewed as favourable management systems for livestock production from the public point of view (Matthews 1996). Animals kept in extensive environments have been shown to have less incidence of contagious disease than those kept in intensive environments (Lawrence and Appleby 1996). However, extensive environments have some other inherent challenges to sheep health such as increased susceptibility to predation (Manning *et al.* 2014) and exposure to extremes of heat and cold (Done-Currie *et al.* 1984; Marai *et al.* 2007) among others. Illness

and disease may go untreated for long periods of time, resulting in poor health outcomes for the animal.

2.2.4 Physiological measurements

There are numerous methods available with which to measure physiological aspects of sheep health. Some measures, such as taking rectal temperature, can be invasive to measure to varying degrees, and may introduce new stressors through restraint and handling (Chung *et al.* 2010). Mendl (1991) has questioned the usefulness of physiological measurements alone as an indicator of physical wellbeing, especially given the difficulty in ascertaining an accurate cut-off point to determine good or poor health. However physiological parameters overwhelmingly are associated with many illnesses and diseases of sheep, and the ability to measure them in the field is therefore an important tool in the management of health and wellbeing issues in livestock.

Physiological measures of health can help to provide an objective health profile. Ailments such as fly strike have been observed to induce physiological changes which are characteristic of an acute phase response, often associated with tissue damage, stressors and infection (Colditz *et al.* 2005). In order to objectively and quantitatively measure an animal's response to illness or disease an automated system must be able to detect the parameters commonly associated with known health issues, this would enable more timely treatment, particularly in the case of conditions in which behavioural changes are only exhibited in the advanced stages due to the reputation sheep have for appearing "stoic" (Dwyer 2008).

Another physiological measure of health is haematology, which can be used to indicate infection or illness. Elevated white cell counts and other immunological indicators, in addition to plasma cortisol, are associated with a wide variety of bacterial, parasitic, viral and physiological conditions including but not limited to: heat stress (Srikandakumar *et al.* 2003; Alhidary *et al.* 2012), mycobacterial infections (Sharp 2008), and liver fluke (Mitchell 2008). Blood sampling in automated monitoring is not possible at this stage due to these procedures being invasive and requiring close handling, and are therefore not currently able to be automated or performed remotely.

Another physiological parameter which may indicate a health issue is respiratory rate. For example, this is increased in sheep experiencing heat stress (Srikandakumar *et al.* 2003), and can be measured using a stethoscope but again this requires close handling of the animal, additionally the presence of wool may interfere with this measurement. Similarly, heart rate

increases are associated with a wide range of conditions including hypothermic stress (Panaretto and Vickery 1970), and this measure is often performed at the same time as respiration measurements using a stethoscope with close handling of the animal.

One physiological measure with a number of different measurement methods available is that of body temperature. Body temperature changes are associated with a number of illnesses, ectoparasite rickettsial infections such as those associated with tick fever (Woldehiwet 2008) and fly strike (Colditz *et al.* 2005); environmentally induced problems such as failure to maintain heat balance during heat stress (Marai *et al.* 2007), dehydration (Saini *et al.* 2013); and viral infections such as Bluetongue (Osburn 2008). Body temperature is a clinical sign which is often used in a diagnostic capacity for a large range of illnesses. However, there are some complicating factors in the measurement of temperature in sheep, as most measurement devices have traditionally required close handling (Goodwin 1998) and some equipment used, such as rectal probes, may introduce risks of disease transmission (Katsoulos *et al.* 2016). In the following section, common diseases and their physiological and behavioural indicators will be explored.

2.3 Common health issues and indicators of health in sheep

In Australia, there are a number of common issues pertaining to sheep health and wellbeing concerning producers and consumers (Dwyer 2009; Windsor and Lomax 2013). Addressing these issues has become a priority for industry, and has prompted research into identifying health problems, with a view to addressing these. Many of these health issues are significant as in the extensive production system, monitoring of animals can be infrequent, and by the time conditions which began as acute are observed, they may have progressed into a more chronic illness. When investigating these health issues, particularly in extensively raised sheep, it is important to identify which measures would be useful and applicable in an extensive environment. Measurement needs to be objective, and preferably achievable using currently available resources, which can be implemented in a remote and automated way. Measurements which can be performed in a minimally invasive way can reduce the potential for stress during monitoring for health assessment purposes.

2.3.1 Lameness

Lameness is considered to be a significant problem in sheep production systems (Winter 2008), and can be attributed to a number of causes; terrain, wetness, injury, hoof overgrowth, limb conformational abnormalities and inflammation (Bokko *et al.* 2003). Lane *et al.* (2015) attributes significant economic losses to the Australian sheep industry resulting from foot abscesses (\$10M), and footrot both virulent (\$32M) and benign (\$12M). Lameness is usually identified visually and scored according to severity (Colditz *et al.* 2011). Improved management practices can contribute to reduced incidence of lameness (Winter *et al.* 2015), similarly, treatment with antibiotics for footrot results in reduced lameness (Kaler *et al.* 2010). In addition to footrot, other diseases can cause lameness, such as joint infection (Scott and Sargison 2012), contagious ovine digital dermatitis, scald, white line disease, and toe granulomas; regular monitoring and treatment of these issues can help to resolve many cases of lameness (Hodgkinson 2010). Lameness, whether caused by disease or some other pathology, can threaten the overall health of an animal. Observations of altered circadian patterns in the rectal temperatures of sheep affected by foot rot suggests a link between the ability of the animal to regulate its temperature according to circadian patterns and the pathology of the foot rot (D'Alterio *et al.* 2012).

2.3.2 Predation

Predation by wild dogs, dingos, foxes and pigs is an increasingly significant health issue for sheep in Australia and around the world, particularly in extensive production systems where continual monitoring is difficult (Choquenot *et al.* 1997; Mattiello *et al.* 2012), and can result in both physical injuries as well as the ongoing impact of repeated stressful events (Manning *et al.* 2014). Injured animals may go undetected and untreated for some time, as the only apparent indicator of an attack is the visual identification of obvious injuries. Delay in identification and treatment may lead to secondary complications such as bacterial infection and the ability to maintain homeostasis, including internal temperature regulation, may be compromised.

2.3.3 Parasites

Parasites are an important health and wellbeing issue in extensively raised sheep. Internal parasites cost the sheep industry an estimated \$435M annually, second only to peri-natal mortalities in economic costs in Australia (Lane *et al.* 2015). *Haemonchus contortus* (barbers pole worm) is a common internal parasite of sheep raised in high summer rainfall areas in Australia, it infects the abomasum of ruminants and causes anaemia in affected animals (Vlassoff and McKenna 1994). Signs of parasite infection may include dehydration, anaemia, decrease in weight gain, lethargy and more (Jackson and Coop 2008). High parasite loads can result in immunocompromised animals, leaving animals susceptible to secondary bacterial or viral infections. Gastrointestinal helminths are a significant problem, with many species of parasite invading the gastrointestinal systems of sheep. Commonly treated with anthelmintic formulations, these parasites can become resistant, resulting in the need for careful management of this sheep health risk (Jackson and Coop 2008).

Lice are a significant ectoparasite of sheep, causing itching and discomfort as a result of feeding and movement (Levot 1995), and costing the Australian sheep industry an annual \$81M (Lane *et al.* 2015), early signs of infestation are often subtle, such as rubbing on fence posts (Bates 2008). One of the most significant problems associated with ectoparasites in sheep is fly strike (cutaneous myiasis). This condition is the result of larval infestations of the sheep blowfly *Lucilia cuprina* and is associated with many systemic effects, including high body temperature, and can be a significant problem in extensive sheep production (Phillips 2009; Wall and Ellse 2011). Agitation is one of the early signs of fly strike, and animals may show stamping behaviour and rubbing depending on the location of the strike on the body, discolouration of

the fleece and an associated odour are also signs of flystrike infestation (Bates 2008). The ability to detect and treat fly strike at an early stage would be advantageous to producers and improve the well-being of stock. Currently producers usually detect fly struck sheep when they are handled for other husbandry procedures such as weaning, crutching and shearing. This is usually done by sighting patches of wool affected by exudate from infected skin, itching behaviour, and the smell of the affected area on the sheep; however the struck areas can be well advanced by the time an infected sheep is detected (Cramp *et al.* 2009). There are currently no commercially available technologies which allow for remote automated detection of fly strike in sheep. Proof of concept has been demonstrated with an electronic nose in the detection of odours associated with strike, further investigation as to its usefulness in the field is needed (Cramp *et al.* 2009). Sustained infections with fly strike are associated with a range of signs including systemic toxemia which results in an increase in core body temperature (Walkden-Brown *et al.* 2000; Colditz *et al.* 2005; Phillips 2009; Horton *et al.* 2017), due to the inability of the animal to maintain its thermoregulatory processes while battling a chronic infection of the ectoparasite.

2.3.4 Other disease

While prevention of disease has been identified as preferable to treatment after disease has taken hold (Roger 2008), where disease does present treatment should be carried out as soon as possible to increase the likelihood of a successful outcome. Sheep in extensive environments are exposed to numerous diseases and illnesses. Some of the most common of these illnesses include clostridial bacterial infections such as blackleg, the symptoms of which include fever and stiff gaits, and tetanus which is also characterised by stiffness which increases to convulsions (Lewis 2008). These diseases cost the Australian sheep industry around \$31M annually (Lane *et al.* 2015). Septicaemia may also result from untreated wounds and bacterial infections which have spread. Bluetongue is a viral disease most often characterised by fever (Osburn 2008). Ovine Johnes Disease is another significant pathology of sheep in Australia (Animal Health Australia 2012), costing an annual \$34M in production losses, prevention and treatment costs (Lane *et al.* 2015). Other diseases affecting sheep health in Australia include pregnancy toxemia (Sargison 2008), mastitis (Watkins and Jones 2008), perennial rye grass toxicoses (Lane *et al.* 2015) and many more. Several of these bacterial infections of sheep are associated with a change in body temperature, as the body fails to maintain homeostasis of the internal environment.

2.3.5 Husbandry practices

Many routine husbandry practices can impact on the health and wellbeing of sheep. There is some evidence to suggest that the routine practice of shearing can result in stress-induced hyperthermia (Sanger *et al.* 2011), along with other physiological stress responses (Hargreaves and Hutson 1990a), suggesting that shearing causes some issues with health, related to both the handling and procedure, and the effects of the removal of wool. Wool removal has shown a significant effect on the heat loss and core temperature of sheep under a range of environmental conditions (Beatty *et al.* 2008a). Health issues can occur during lambing, requiring treatment and additional handling, complications such as prolapse (Hosie 2008) and dystocia (Hindson and Winter 2008) increasing the risk of illness and injury to the ewe.

In addition to husbandry practices common to sheep production in Australia, it is inevitable that in extensively raised sheep human interaction may be infrequent and therefore stressful to the animals, with the addition of dogs for herding producing an additional source of fear (Dwyer 2009). Handling is often necessary to diagnose and treat illnesses, as well as check for early signs of illness. Issues associated with handling include transport. Transport of sheep to different climatic regions can influence the health and wellbeing of animals, measures such as shearing prior to a voyage can prevent suffering due to heat stress (Beatty *et al.* 2008a). Therefore, the impact of handling on extensively raised sheep or those which are unaccustomed to handling should be considered when developing a health monitoring and management system.

Stockmanship has been identified as a critical factor in the management of intensively raised animals, both in the improvement of sheep health and wellbeing (Hemsworth *et al.* 2009) and in productivity (Hemsworth *et al.* 2000). While in the past signs of stress have been limited to behavioural observations and direct measurements of blood chemistry (Hargreaves and Hutson 1990b), the measurement of eye temperature is becoming a new method of detecting indicators of stress (Stewart *et al.* 2008a). Therefore, any health measurement system which involves handling should take these important aspects into account. Extensive sheep production systems are common in Australia, with sheep predominantly grazed rather than fed except during times of drought. Animals kept in extensive environments have been shown to have less incidence of contagious disease than those kept in intensive environments (Lawrence and Appleby 1996), but extensive environments have some other inherent challenges to health. Some issues may arise due to the difficulty in maintaining regular inspections of stock, increased susceptibility

to predation (Manning *et al.* 2014) and exposure to extremes of heat and cold (Done-Currie *et al.* 1984; Marai *et al.* 2007) to name a few.

2.3.6 Indicators of common health issues

Many of the health issues addressed in the above sections have several commonalities in their signs and symptoms. Fever, inflammation, ill-thrift, lethargy, weight loss and anorexia and many more (Lovatt 2010). Many of these indicators rely on close handling and/or invasive or semi-invasive measurements in order to assist with a clinical diagnosis, and also may require veterinary assistance to aid in diagnosis. Others, such as temperature measurement, are easy to measure and generally adhere to well defined parameters of normal and abnormal levels. In the following section, this parameter will be investigated more closely.

2.4 Temperature measurement as a health indicator

2.4.1 Thermoregulation

Thermoregulation involves the ability of animals to regulate their internal temperature, and is important for the healthy maintenance of body systems. Many of the catalytic reactions which occur in the body require a consistent temperature range in order to progress. When an animal's internal temperature changes significantly, it can lead to reductions in cell viability (Romero *et al.* 2013) and has flow on effects on the health of the animal (Silanikove 2000). There are aspects of extensive production systems in which the health of animals may be at risk, such as from weather extremes, disease and injury (Matthews 1996). The ability of the animal to regulate its internal temperature may be compromised by several conditions both from within and without, due to either direct attack from a pathogen, or the excessive use of energy and immune resources employed by the animal to combat the effects of the disease (Colditz 2002; Roth *et al.* 2006) or the environment (Panaretto and Vickery 1970).

2.4.1.1 Key characteristics of sheep thermophysiology and thermoregulation.

Sheep have some characteristic mechanisms of thermophysiology which make them unique among many other domestic species. Many of these mechanisms are a consequence of their ability to grow wool. For example, a Merino sheep grows wool vertically in a compact insulating layer, this layer is able to maintain a temperature differential of up to 40-50°C between the body temperature of the animal and that of its environment depending on the density, thickness and distribution of the wool (MacFarlane 1964). This insulating layer improves the animal's ability to both retain body heat during periods of cold ambient temperatures, and hinders their ability to lose heat during periods of high temperatures.

The change of an animal's internal temperature may be the result of factors from within (disease), without (environment), or both. Many diseases of sheep can result in the activation of the febrile process in order to combat the effects of the pathogen (McKinley *et al.* 2011). Additionally, many common practices, such as shearing, can expose the animal to environmental conditions which may also challenge the sheep's ability to maintain its body temperature, due to excessive heat loss such as that experienced in cold and wet conditions (Panaretto and Vickery 1970), or due to the inability to lose heat, such as experienced during heat stress (Marai *et al.* 2007). Consequently, it is considered that a change of diagnostic significance in body temperature is indicative of compromised health of the animal (Fielder 2017).

There are a number of physiological processes which can be employed by sheep to enable them to cope with their environment and maintain their internal temperature. One of the thermoregulatory processes available to sheep is selective brain cooling, a process by which blood is diverted via the carotid rete, a collection of blood vessels in the venous cavernous sinus, prior to arriving at the brain, resulting in blood supplying the brain becoming cooler than that supplying the rest of the body (Kuhnen 1997; Caputa 2004; McKinley *et al.* 2011). This usually occurs at high temperatures and performs the dual function of conserving water and minimizing thermal damage to the brain (Strauss *et al.* 2015). Selective brain cooling is also implicated in other thermoregulatory processes in sheep. For example, dehydration has been shown to increase the magnitude of selective brain cooling in sheep, irrespective of the core temperature of the animal (Fuller *et al.* 2007).

Selective brain cooling in sheep has implications for the effective measurement of body temperature. Measurement locations close to the carotid rete may be affected by interference of this cooling mechanism and therefore may not offer a true representation of the body temperature of the animal, even showing a difference from one side to another (Kuhnen 1995). Additionally, the ability of selective brain cooling to offset the effects of high environmental temperatures may present a false impression of acceptable environmental temperatures, as the animal is undertaking a thermoregulatory process to mitigate the potential neurological damage posed by the excessive temperature, even though the other body systems may be suffering the effects of excessive heat load (Nagasaka *et al.* 1998).

Sheep can also experience hypothermic stress when exposed to cold, wet environments after shearing (Panaretto and Vickery 1970). When investigating the effects of cold on sheep, loading the rumen with cold water (0-1°C) results in a change of behaviour, and can result in sheep exhibiting thermoregulatory behaviours such as those observed in sheep trained to press a panel to obtain infrared heat (Baldwin 1975). While the effect of cold on thermoregulatory processes is not generally associated with illness or disease, the inability of an animal to regulate its temperature through adverse weather conditions may indicate the failure of these inherent processes, which in turn may be indicative of the presence of disease or illness.

2.4.1.2 Challenges to thermoregulation

There are several disease states in animals which can impact on the regulation of an animal's internal environment. This may occur as a result of either direct attack on body systems invoking a febrile response (Blatteis *et al.* 1987), or the drain on resources needed to combat

the effects of the illness, thus limiting the resources available for temperature regulation - this is known as a metabolic cost (Colditz 2002). For example, stress and dehydration and their impact on the ability of sheep to regulate temperature has been investigated, and core (rectal) temperature increases during periods of dehydration are believed to be related to the reduction of skin blood flow and sweating rate (Saini *et al.* 2013). Heat stress has also been shown to be associated with an increase in rectal temperature (Srikandakumar *et al.* 2003).

Fever is identified as a critical indicator of response to most infections, and is activated and regulated by a number of immune system components. This activation can occur due to exposure to agents such as bacterial endotoxins, for example lipopolysaccharide (LPS). As a result of this activation LPS itself can be used to model fever in animals (Godfrey *et al.* 2007; Smith *et al.* 2011; Harden *et al.* 2014). Elevated body temperature is a clinical sign for many diseases of sheep, including tick fever (Woldehiwet 2008), advanced fly strike (Colditz *et al.* 2005) bluetongue virus (de Diego *et al.* 2013), and other viral infections (Osburn 2008).

The investigation of body temperature as a measure of animal health therefore, must account for the interactions and influences of the thermoregulatory processes in the body. There exists a degree of natural variation both between individuals, and within an individual, the latter being attributable to circadian or diurnal patterns of body temperature (Maloney *et al.* 2013). Therefore, it is important that when measuring temperature with a view to utilising it in a diagnostic capacity it has the ability to detect changes in body temperature outside of the normal variation, and at the level of the individual animal.

2.4.1.3 Diurnal rhythmicity of body temperature

Body temperature patterns follow a daily oscillation rhythm, with temperatures falling through the night and rising during the day (Refinetti and Menaker 1992). This rhythmicity can be altered by a number of factors, such as feed intake (Maloney *et al.* 2013), illnesses (D'Alterio *et al.* 2012), metabolic processes (Haydon *et al.* 2013), rumination (Tebot *et al.* 2009), age of the animal (Piccione *et al.* 2002b), shearing (Piccione and Caola 2003) and many more. Changes attributable to daily rhythmicity of body temperature have been observed in foot temperature measurements in sheep with the use of infrared thermography, although the oscillations observed were less robust (i.e. temperature variation was reduced) than core temperatures recorded as rectal temperature, and amplitude (the difference between the mean value and the peak of the rhythm) differed from core body temperature rhythm (D'Alterio *et al.* 2011).

External factors can impact on diurnal patterns. For example, in a cold environment, the amplitude of the variation in body temperature in sheep has been shown to increase when compared to that in thermoneutrality (Piccione *et al.* 2013a), and shearing can decrease the amplitude (Piccione *et al.* 2002a). Additionally, season and physiological status has also been found to affect circadian rhythm of body temperature in cattle, both in terms of the amplitude and the period of rhythmicity (Kendall and Webster 2009). Change in rhythmicity can also be indicative of specific health issues; for example, the presence of fever has been found to eliminate the observable circadian pattern of rectal temperature in sheep suffering from foot rot (D'Alterio *et al.* 2012), indicating that not only do some illnesses cause changes within the rhythm, but also there is the potential to remove any observable rhythm.

2.4.2 Traditional methods of temperature measurement in sheep

2.4.2.1 Rectal and vaginal temperature measurement

There are a number of ways to measure temperature in animals; one of the most commonly used measures of internal temperature for sheep and other mammals is rectal temperature. It is a relatively stress free procedure if the animal is accustomed to handling, and is considered one of the most practical and accurate measures to undertake in the assessment of animal health. It is usually taken with a spirit or digital thermometer. However, rectal temperature can vary due to a number of factors, such as digestion, faecal masses, and physical activity (Abecia *et al.* 2015) while the proximity to the skin surface and the thermoregulatory processes associated with blood flow to the skin and mitigation processes such as sweating (Marai *et al.* 2007) can also have an effect on this measurement. As a result, different methods of temperature measurement have been investigated, in order to determine both the measurement location and method most likely to reflect “core temperature”, and the ability of these measures detect significant deviations from the “normal” range, thus broadening the applicability of these measures to extensive systems.

Where rectal and vaginal temperature measurements have been compared, they have been highly correlated in sheep and cattle (Suthar *et al.* 2013; George *et al.* 2014). However, when measuring temperature over a period of time, vaginal temperature may provide a more consistent measurement since rectal temperature may be disrupted with the movement of fecal matter. Additionally, vaginal temperatures are only of use in female animals.

The main drawbacks to the use of traditional rectal thermometers are that they usually require handling and restraint of the animal, and handling which has been identified through this review

as a source of stress and is itself capable of influencing body temperature measurements. This is particularly problematic in the case of extensively raised animals which are usually handled infrequently and are unlikely to be accustomed to being restrained.

2.4.2.2 Variability in temperature measurement

An important aspect of temperature regulation, as it relates to measurement of temperature in sheep, is the diurnal pattern which is observed in many animals. These circadian rhythms of a strict 24-hour cycle have been observed in sheep with deep body temperatures observed at their lowest in the early morning (Gordon and McAllister 1970), and their highest in the late afternoon (Saidu *et al.* 2012). These diurnal temperatures can vary up to 2°C in healthy sheep (MacFarlane 1964). Rumination is closely associated with light sleep in ruminants, and the pattern of sleep varies according to the circadian rhythm in response to light cycles (Gordon and McAllister 1970). As a result of this normal and natural variation in body temperature, any measurement system must incorporate the capacity to differentiate between normal variation within the diurnal pattern, and body temperatures occurring outside of the normal range. Temperature sensors embedded in rumen bolus' have shown some accuracy in measurements in cattle studies (Ipema *et al.* 2008), however the rumen environment is different to many other organs of the body due to the activity associated with the rumen ecology (Beatty *et al.* 2008b), and the heat of fermentation, and is therefore also subject to unique sources of variability. Consequently, rumen temperatures are often higher than rectal temperatures (Piccione *et al.* 2013a).

The consequence of this variation lies in the difficulty in establishing “set points” or thresholds for temperature ranges considered to be a result of fever, and the definition of fever varies somewhat. Some researchers characterized the onset of fever as a rapid decrease in temperature along with visible shivering (Blatteis *et al.* 1987), while others characterize it as a rise in body temperature along with a re-adjustment of the set point due to the persistence of thermoeffector activities as opposed to rises characterized as hyperthermia (Roth *et al.* 2006), while yet others establish a definition based on a maximum temperature which is consistent with fever in that species (de Diego *et al.* 2013). Due to these differences, it is important to investigate the mechanism of fever as it pertains to the species of interest and investigate the thresholds at which an animal may be determined to be “feverish”.

2.4.2.3 Influences on temperature measurement

There are several external influences related to body temperature in sheep. An example of this is the effect of diet; there is some evidence to suggest that dietary influences on body temperature may be observed. For example, the concentration of crude protein in the diet has been observed to affect body temperature. The influence of this appears dependent on the time of day (Ahmed and Abdelatif 1995). This has important implications for the measurement of temperature in extensively raised animals which may have a high variation in diet compared to more intensive systems where dietary intake is more controlled and consistent. Additionally, the respiratory rate is variable between different breeds of sheep and is an important aspect of temperature regulation, the nasal cavity acting as an evaporative cooler in warm ambient temperatures (MacFarlane 1964). It is this variation which is important to consider when looking at locations on the animal for temperature measurement as these physiological aspects impact on both the absolute temperatures that an animal experiences, and the rate and amplitude of change which may be experienced under both normal and pathological states. Measurement of temperature in absolute values is difficult due to variation between animals, as well as the normal variation that occurs when the body changes to maintain homeostasis (Barnett and Hemsworth 1990). Therefore, it is important to determine the extent of change within an individual of a parameter such as body temperature, which may indicate a pyrogenic response to illness or disease.

When considering the above influences on core body temperature, it becomes evident that a method of temperature measurement needs to incorporate the ability to establish a baseline range for individual animals, and therefore it can be assumed that sporadic measurements will be less useful in this capacity than more consistent measures which can be collated and analysed statistically to enable the development of “normal” thresholds and patterns for each animal, allowing abnormal conditions to be more easily identified. With this in mind, the following section will investigate the possible options for the development of remote body temperature monitoring.

2.5 Monitoring body temperature

2.5.1 Temperature measurement in sheep health monitoring

Sheep production in Australia is largely extensive, making traditional body temperature measurement methods impractical for health assessment in sheep. Yet real time body temperature measurement has been identified as an important dimension in remote health monitoring systems (Morris *et al.* 2012). Therefore, it is important to identify ways in which these barriers can be overcome, so that this important health measurement parameter can be utilised in these extensive production systems.

2.5.2 Remote temperature measurement technologies

The ability to remotely monitor body temperature would assist producers in the health monitoring of their stock, disease management and illness prevention. The availability of the new technologies opens up a new perspective and application for many existing technologies which could be optimised for use in the animal production industries. The most promising of these are discussed in the following section.

2.5.2.1 Temperature loggers

There are currently several temperature measurement technologies available which may be useful in the monitoring of body temperature in sheep. For example, temperature sensing data loggers such as ThermoChron iButtons® (Langer and Fietz 2014) can be a useful method of measuring body temperature, these devices combine a thermometer, data logger and battery in a small (19mm diameter) unit. These devices have some limitations in their usefulness in the field as they rely on battery power and must be removed from the animal in order to download the information, so measurements cannot be observed in real time, reducing the ability to identify potentially ill animals. iButton® devices must therefore be either surgically implanted or placed intravaginally, and blood supply to the area is important in determining the effectiveness of the implant site in approximating a core body temperature. As a result of these factors iButtons® and similar loggers have limited capabilities in a remote and automated temperature measurement system.

2.5.2.2 Infrared thermography

Infrared thermography has significant potential in the investigation of non-invasive methods of measurement of body temperature in animals. Thermal energy has a much longer wavelength than visible light, whereas in thermal imaging the spectrum which is seen is

expanded, enabling us to see and measure thermal energy emitted from an object (FLIR 2016). Infrared thermography has been the subject of a great deal of research, showing utility in the early detection of inflammation (Ju *et al.* 2005; Poljak-Blazi *et al.* 2009) and disease (Schaefer *et al.* 2004; Stewart *et al.* 2005; Rainwater-Lovett *et al.* 2009). George *et al.* (2014) found a moderate correlation between vaginal temperature (measured by digital thermometer) and eye temperature as measured by infrared thermography. Eye temperature can be an effective indicator of elevated body temperature, however will not diagnose subclinical or localized infections such as those seen in flystrike (George *et al.* 2014). Infrared thermography measurement of body temperature is potentially limited by its non-invasive nature, in that it will detect the thermal energy emitted by a surface but this is not always an accurate reflection of internal body temperature, and therefore more work must be done to determine the most useful application of this technology.

Mastitis detection in sheep has been investigated with the use of infrared thermography. Infrared udder temperature can be used as a diagnostic tool to detect mastitis in sheep. It is difficult to know whether this technology could be applied to other illnesses or injuries due to the presence of wool over much of the rest of the animal's body, the udder has little covering to insulate the area (Martins *et al.* 2013). Infrared thermography shows promise in the area of automated temperature monitoring, however, its utility and accuracy in remote monitoring is impacted by fur or wool, which vary in their thermal exchange properties, covering the area of interest (Cena 1973). The infrared signal is impacted by moisture which impacts on the ability of the sensor to detect and accurately measure the thermal energy emitted from a surface.

The application of infra-red thermography of eye temperatures in the detection of illness and disease in sheep in a remote monitoring capacity is not currently known, however it is a measurement method which may have potential in the area of remote temperature monitoring in sheep.

2.5.2.3 Ear tags

Temperature sensing ear tags are a new technology which has been introduced initially in cattle and dairy to detect illness and oestrus in cattle. It is a device in which a temperature probe is attached to an ear tag logging device and the probe extends into the auditory canal of the animal (Anonymous 2010). The usefulness of temperature sensing ear tags in sheep is yet to be investigated; however, it is expected that weight and battery life would need to be improved before they could be seen as a practical solution in extensively raised sheep.

2.5.2.4 Microchips

A temperature measurement device which has clear potential in the application of remote monitoring systems is the temperature sensing LifeChip® with BioThermo™ (Destron Fearing 2009). This is a relatively recent technology with a high degree of potential for utility for animal health monitoring in extensive environments, given that much of the needed infrastructure in terms of readers is already in use in the sheep industry. The device comprises a traditional radio-frequency identification (RFID) device combined with a temperature sensor, when the microchip receives a small electrical pulse from a reader (usually a wand) it transmits a unique RFID number and a temperature reading.

While RFID systems have been in use for some time in many animal industries, from livestock identification tags to companion animal identification, they are increasing in acceptance and capabilities. Some of their current applications in commercial use involve utilising RFID technology in tracking animals and matching progeny (Mustafa *et al.* 2014), such as Pedigree Matchmaker (Cooperative Research Centre for Sheep Industry Innovation), these systems involve the basic RFID microchip without a temperature sensor.

2.5.2.4.1 Temperature sensing microchips in detection of febrile animals

One example of the application of temperature sensing microchips in a measurement capacity was the recording of temperatures of Thoroughbred foals during an outbreak of Equine encephalitis (Grewar *et al.* 2015) and pyrexia was defined as foal body temperature of 39.9 degrees Celsius or a body temperature greater than 1.96 standard deviations above the mean of six previous readings for that individual. It is this application which has the potential to be useful to extensive sheep productions in the identification of pathologies which have a diagnostic parameter of body temperature change.

2.5.2.4.2 Placement of microchips

A study by Kort *et al.* (1998), has examined the use of temperature sensing transponders to measure body temperature in rats with results indicating they may be useful in this application. The findings also showed there were differences in the measurements depending on the placement of the microchip transponder (subcutaneously compared with intraperitoneal). Additionally, RFID temperature sensing microchips have been trialed in horses, with results showing that the implantation site selection is an important factor in the reliability of the results (Marsh *et al.* 2008).

There are several influences on body temperature at different locations on the body, while the tail is often used for implants due to ease of access and low post-slaughter value, it is also influenced by activity levels, movement of faeces, and the circulation to the extremities which can confound a body temperature measurement. Capillary blood flow has been shown to be more heavily affected by local temperature effects than signals from the hypothalamus, demonstrating that a thermal gradient across the skin is involved with different thermoregulatory processes (Hales *et al.* 1978). Additionally, dehydration limits cardiovascular and thermoregulatory responses, resulting in an increase in body temperature (Saini *et al.* 2013). Locations near the head are also subject to both exogenous and endogenous thermal influences which may impact on the ability of the microchip to reflect core temperatures, one such influence is selective brain cooling, in which blood flow is diverted through the carotid rete in order to cool the blood supply to the brain, and this mechanism has been shown to be initiated and maintained during fever (McKinley *et al.* 2011). It is not known whether this would impact on the accurate measurement of body temperature at locations on and around the head of the animal. Locating a measurement device on an ear may also be subject to external thermal influences, such as radiant heat from the sun, as well as the thermoregulation of the ear in which blood vessels are located close to the skin surface in order to facilitate heat exchange. These thermoregulatory responses which have been observed in sheep can be used to identify implant locations which are most likely to accurately reflect core temperature changes in a sick or injured animal, or rule out areas which may be considered unlikely to be representative of core temperature changes.

One of the limitations of these devices is that early variants of the RFID microchip have been known to migrate when implanted subcutaneously, with some implant sites varying significantly in the distance they had migrated (Caja *et al.* 1998), this is problematic when it comes to utilising panel readers in the field, which rely on consistent orientation of the device to the antenna to get accurate reads. Manufacturers have sought to address this problem by coating the microchip in substances which bind to connective tissue in the animal, limiting the migration of the microchip over the life of the animal (Destron Fearing 2009).

Experiments in sheep have been performed to ascertain the usefulness of these devices in the detection of core body temperatures in sheep. Stress was used to induce these temperature changes, and measurements were taken comparing subcutaneous microchip implants located under the tail, in addition to vaginal measurements by iButton® and ocular temperature through infrared thermography. The results showed that subcutaneous and IR temperature readings

underestimate internal temperatures (Abecia *et al.* 2015). Further investigation is needed to accurately assess these devices in terms of detecting illness and disease in sheep in the field.

2.5.2.4.3 Practical extensive application of microchips in sheep

The usefulness of temperature sensing microchips may be compromised by their inability to accurately reflect the absolute temperature of an animal, as their accuracy is only $\pm 1^{\circ}\text{C}$ (Destron Fearing 2009). However, given the significant differences in baseline temperatures between individuals, it is possible that temperature sensing microchips have the ability to detect a change in temperature outside of what is normal for that animal. The advantage of this method is that it enables a baseline for individual animals to be established over a relatively quick period of time through the positioning of a reader, to allow the detection of microchip data on a daily basis. By detecting temperatures frequently during normal activities, such as moving to a water point, a body of data for each animal can be maintained, making it easier to identify a body temperature outside of that normally associated with that animal.

The application of RFID technology has had little research in extensive management systems. The potential application of temperature sensing microchips as part of a remote monitoring system for the health and wellbeing of sheep in extensive production systems is significant. The ability to monitor body temperature in a remote and automated way would increase the capacity for these systems to monitor the health and wellbeing of their animals in a more consistent and regular manner, building a health profile for each individual animal and enabling the rapid detection and treatment of sick and injured animals, improving the health outcomes of sheep in extensively raised production systems.

2.5.3 Overview

The area of remote health monitoring in sheep has significant implications for the future of sheep production in Australia and globally, with new technologies becoming more available and affordable all the time. As more of the barriers are addressed, such as network capability and integration, it is evident that these technologies will play a larger role in the health management of extensively raised sheep. Measurement technologies also continue to become smaller and more efficient, allowing their application to flow through to the small ruminant sector. The role of body temperature measurement, therefore, is a critical one as producers broaden their capacity to monitor the health of their animals in a remote and automated way.

2.6 Summary

2.6.1 Summary of literature review

Current knowledge covered in this literature review suggests that extensively raised sheep are at risk of poor health and wellbeing despite the increased freedom of movement and behaviour (Dwyer 2009) when compared to intensive production systems. This is considered a consequence of delays in treatment, increased susceptibility to climate, predation and food and water provision variability. It has been outlined that it is difficult to maintain continuous monitoring in extensively raised sheep, but established and emerging technologies may be able to offer promise in the application of a remote monitoring system. It is currently not known which of these technologies would be most useful in the detection of ill and injured sheep in extensive production systems.

The development of a remote automated monitoring system for detection of body temperature in extensively raised sheep would enable producers a useful diagnostic tool and early warning system for a range of health and wellbeing issues associated with sheep production. The ability to monitor and detect significant temperature changes for individual sheep allows changes which may be associated with pathology to be flagged and acted upon earlier than they would otherwise be if relying on traditional methods of illness detection in extensive production systems. A remote monitoring system incorporating temperature measurement technology would significantly enhance the capabilities of producers in monitoring their livestock in real time, whilst minimising both the cost of labour intensive monitoring and the stressful interactions which often occur during handling.

The aim of the experimental work in this thesis is to identify a quantitative measure of animal health which is simple to implement and uses currently available technology which can then be integrated into a remote monitoring system. This would allow for a health and wellbeing profile at the level of the individual animal, enabling producers to identify issues at an early stage and respond more quickly with treatment and prevention. Integration of remote temperature measurement technologies assists producers in the prevention of widespread health issues becoming established throughout their flock.

The subsequent chapters will attempt to answer the following questions:

1. Do peripheral temperatures as measured by microchip temperature sensors, detect circadian rhythms and reflect core temperature measurements?

2. Does the location of a microchip temperature sensor have an impact on the ability of the sensor to detect changes in body temperature which may be of clinical significance?
3. Does infrared thermography offer the possibility of the detection of inflammation associated with fly strike in fleeced sheep?
4. Do these technologies have the capacity to be utilized under field conditions as part of a remote and automated animal health and wellbeing monitoring system?

Chapter 3

Daily rhythmicity of body temperature and peripheral temperature measurements in sheep.

Abstract.

Understanding circadian rhythms of body temperature is important in the interpretation of body temperature measurements and assessment of physiological state of an animal. The ability to measure body temperature at peripheral locations may also be important in the development of minimally invasive tools for remote temperature measurement in livestock, providing an indication of the health status of an animal. This study aimed to investigate how well body temperature measured at peripheral sites reflected core (vaginal) temperature (T_v) detection and the daily rhythmicity in the body temperature of sheep. The impact of feeding frequency on both core and peripheral temperature measures was also investigated. Eleven crossbred ewes were implanted with peripheral temperature sensing microchips (LifeChip®) which were positioned transversely in the sternocleidomastoid (neck) muscle (T_n) and subcutaneously under the tail (T_t). Additionally, iButton® temperature loggers were placed intravaginally to record core body temperature measurements (T_v). Temperature measurements were taken manually at 30 minute intervals during the day from the microchips and automatically from iButtons® set to record temperatures at five minute intervals during the day and overnight for four 24 hour periods. Animals were fed their daily ration in 1, 2, 3 or 5 feeding bouts per 24-hour period. The body temperature measurements observed for the peripheral sites T_n and T_t differed significantly to those measured at the core site, T_v ($P < 0.05$), with T_n lower than T_v and T_t lower than both T_v and T_n . However, pattern similarities were observed across the day between T_v , T_n and T_t in repeated measures analysis, with a short period of difference between T_v and T_n (from 14-16 hrs) and a long period of difference between T_v and T_t (from 10-21 hrs) ($P < 0.05$). Significant differences were found across feeding schedules when analysing circadian rhythm parameters of vaginal temperatures ($P < 0.05$) with peaks and range of oscillation becoming less pronounced as feed schedule increased. These results suggest that further investigation is needed into the potential utility of peripheral measurement devices for use in sheep with respect to determining accurate clinical thresholds relative to the expected normal temperature for the time of day observed. While absolute and fitted temperature parameters differed between T_v , T_n and T_t , pattern similarities were observed at time points across the measurement day, with T_v , T_n and T_t exhibiting similar rhythmicity per the time of day. Additionally, feeding schedule may impact on short term fluctuations over the day in body temperature, but not on overall

rhythmicity and temperature range. These fluctuations may need to be considered when calculating divergence from expected temperatures according to normal circadian patterns.

3.1 Introduction

Circadian rhythmicity of body temperature is a well-known physiological phenomenon describing the daily oscillation of body temperature, elevating during the daylight hours and lowering during darkness. The parameters and characteristics of these oscillations vary between species of mammals (Refinetti and Menaker 1992).

Daily rhythmicity is described by several parameters: the amplitude, which is the difference between the peak (or trough) and the mean value of a wave; acrophase, the time at which the peak of a rhythm occurs; the mesor, which is a circadian rhythm-adjusted mean; and the range of oscillation, the difference between the peak and the trough (Circadian Rhythm Laboratory 2017).

There are several factors which can impact on daily rhythmicity of body temperature. In a cold environment the amplitude of the variation in body temperature in sheep has been shown to increase when compared to that in thermoneutrality (Piccione *et al.* 2013a), and shearing can decrease the amplitude (Piccione *et al.* 2002a). Season and physiological status can also impact circadian rhythm of body temperature in cattle, both in terms of the amplitude and the period of rhythmicity (Kendall and Webster 2009). Change in rhythmicity can indicate specific health issues; for example, the presence of fever has been found to eliminate the observable circadian pattern of rectal temperature in sheep suffering from foot rot (D'Alterio *et al.* 2012). As a result, the ability to observe and analyse circadian rhythms in body temperature and changes from normal rhythm may give insight into a variety of changes in the physiological state of the animal.

To be able to assess differences in body temperature caused by illness, the evaluation of body temperature measurement must also incorporate known normal thresholds for sheep. Core body temperature for sheep ranges from 38.5°C to 39.5°C (Riek *et al.* 1950; Piccione *et al.* 2002a, 2005; Saini *et al.* 2013). When determining the normal temperature for an animal, it is important to consider both the normal core body temperature range, as well as the oscillation which may occur around and within this normal range. Therefore, it is important to not only establish the normal temperature for an animal, but also the normal daily variation.

Feeding is known to impact on the circadian pattern of body temperature in sheep, with temperature gradients within the body, meaning that different measurement sites will have different physiological influences occurring as a result of factors such as heat exchange across blood vessels, and heat from muscular activity. Additionally, the measurements obtained by temperature measurement devices located in different areas of the body may be

influenced by locomotory activity associated with feeding, and metabolic heat production from fermentative digestion of feed (Mohr and Krzywanek 1990). The total intake of feed can also affect circadian patterns. The impact of reduced energy intake was seen in changes in mesor, amplitude and goodness of fit (how well the observed data matches the theoretical model) in body temperature patterns as measured by arterial blood temperature in sheep; fasting animals also showed decreases in maximum body temperature in sheep (Maloney *et al.* 2013).

The factors impacting on core body temperature may not have the same influences on peripheral body temperature measurements; for example, thermoregulatory processes influence the amount of heat leaving the body through blood vessels close to the skin. This occurs by altering blood flow through the arteriovenous anastomoses (AVA) that controls blood flow to the skin, and is influenced by the hormone noradrenaline (NA). These influences, which can be associated with the fight or flight response, or acting in response to heating or cooling influences via the spinal cord, are likely to cause deviations in peripheral temperatures not reflected by core body temperature changes (Hales *et al.* 1978). Therefore, the influence of such physiological factors on core and peripheral temperature measurements means that under different challenges, different thermoregulatory processes may be involved. These could, in turn, influence how accurately peripheral temperature measurements reflect core temperature measurements.

Measurement of body temperature at peripheral locations may be useful in the development and application of remote animal health monitoring systems. Detection and treatment of illness and disease in sheep can be delayed in extensive and other low input production systems due to time and labour constraints (Dwyer 2009), and as a result, animal health and welfare may be compromised (Lawrence and Appleby 1996; Petherick 2005; Goddard *et al.* 2006). Since many common ailments can be alleviated or prevented through timely treatment and management protocols (Roger 2008), the ability to remotely measure key indicators of animal health, such as body temperature change, would potentially be of benefit in such systems.

The development of techniques for measurement of body temperature at peripheral locations can open up a new area of investigation into circadian rhythms of body temperature, and the impacts of various physiological and environmental conditions on the observation and interpretation of this pattern in animals. Measurement of changes in rhythmicity may offer insights into the potential use of peripheral temperature measurement sites for diagnostic outcomes. However, peripheral temperature measurement sites may differ in absolute

temperature. For example, a study comparing core temperature and peripheral temperature undertaken by Mansel *et al.* (2008) in anaesthetised sheep showed that peripheral temperatures that were measured by a thermistor at the ear base and a coronary band did not reflect core temperatures as measured by a thermistor placed in the pulmonary artery of the sheep. Another study by MacLeay *et al.* (2003) found that the subcutaneous peripheral measurement sites of the region of the axilla and proximal inner-thigh were not considered as being representative of core temperature as abdominal and carotid measurement sites in monitoring measures of body temperature in sheep. However, the objective of MacLeay *et al.* (2003)'s work was determining temperature changes consistent with hot flashes, and therefore the criteria for determining the equivalency of the peripheral measures may differ to those which are observed for circadian rhythmicity or clinically significant temperature changes.

Some temperature measurement devices have the potential to provide a minimally invasive tool for the measurement of body temperature at a variety of peripheral locations, and temperature sensing microchips have been identified as a currently available technology which may be useful in this capacity (Destron Fearing 2009). The microchips can remain in place for the life of the animal, do not require batteries or replacement, and they are able to be read by wand or panel readers during drafting and other routine stock management procedures without close handling of the animal. With the advent of walk-over-weigh systems there is potential for temperature sensing microchips to become a significant tool in the development of remote animal health monitoring protocols.

This study had two objectives: the first was to investigate the potential of temperature sensing microchips placed at two peripheral temperature monitoring sites to detect core body temperature patterns; and the second: to determine whether different feeding schedules would impact on the core body temperature rhythm. It was hypothesised that microchips placed in peripheral locations in the neck muscle and subcutaneously under the tail would reflect core body temperature rhythms and that different feeding schedules would result in differences in core body temperature rhythms.

3.2 Materials and methods

Two experiments were conducted on 11 sheep over a period of 14 days, with a rest period of 5 days in between the two experiments. Experiment 1 examined the circadian rhythm of body temperature and the accuracy of peripheral measurement sites when compared to core temperature and patterns of change, and took place over a 48-hour measurement period. Experiment 2 examined the influence of different feeding schedules on core body temperature and patterns of change, and took place over a total of 5 days.

3.2.1 Animals and housing

Eleven crossbred ewes (six tooth, tail docked as lambs with a short tail remaining) were introduced into the animal house 16 days prior to the commencement of treatments and were habituated to handling once a day. The sheep were housed throughout the acclimation period in groups of five or six, and were then introduced into individual pens two days prior to commencement of the experimental period. The sheep were shorn immediately prior to entering the animal house, had been treated for internal and external parasites, were in good health and condition, and free of disease. Mean live weight of the sheep was 61 ± 6.5 kg (mean $\pm$ s.d.).

During the experimental period, the sheep were individually penned (2 x 2 m) and companion animals were always in visual and auditory contact. These individual pens restricted excessive activity by the sheep, apart from activity associated with feeding.

The animal house was well ventilated with wooden slatted floors, automatic waterers were available to the animals at all times, and the pens were cleaned daily.

Core body temperature measurements were obtained with an iButton® temperature logger placed intravaginally (Tv) set to measure at 5 minute intervals, and peripheral measurements were obtained using temperature sensing microchips (Destron Fearing LifeChip) implanted transversely into the sternocleidomastoid muscle in the neck (Tn) after wool had been shorn from the area of implantation; and subcutaneously under the tail (Tt). Topical administration of lignocaine and disinfecting solution was administered prior to implantation and implantation was performed 3-5 days prior to the commencement of the experiment in order to allow localised inflammation to subside. Implantation was performed by experienced scientists (Dr I. R. Godwin and Dr A. K. Doughty) and no sedation or sutures were required

for the procedure. Microchips were measured by scanning with a wand reader (Allflex® RS420) at a distance of <20cm.

The experiment was conducted in early December 2015, and daily temperatures ranged between 5.6 and 29.2°C, and relative humidity ranged from 61 to 95% (Australian Bureau of Meteorology 2015). All procedures were approved by the University of New England Animal Ethics Committee, authority number AEC15-096. The care and use of the animals was in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2013).

3.2.2 Feeding

Animals were fed each day at approximately 0730 h during the acclimation period, and any feed refusals were removed and weighed immediately prior to the next ration being given. The ration was a blend of oaten, lucerne and wheaten chaff offered at maintenance levels at a fixed weight calculated per individual sheep weight (9-12MJ ME/sheep.day) during experiment 1 and for FS1 of experiment 2, and a fixed weight of 1.79 kg DM/sheep.day (19.8 MJ ME/sheep.day, approximately 2x maintenance) for experimental period 2 feeding schedules 2-5.

Diet composition is shown in Table 3.1.

Table 3.1 Feed composition (dry matter basis) for lucerne/oaten/wheaten chaff blend analysis by NIR.

Nutrient Description	Sample
Dry Matter (%)	89.4
Neutral Detergent Fibre (%)	49
Acid Detergent Fibre (%)	29
Water Soluble Carbohydrate (%)	11
Crude Protein (%)	18.3
Inorganic Ash (%)	11
Organic Matter (%)	89
DMD (%)	67
DOMD (%)	64
AFIA Grade A2	A2
Metabolisable Energy (MJ/kg DM)	9.9

3.2.3 Design – Experiment 1: Detection of core and peripheral temperature rhythms

Sheep were offered their entire ration at 0730 h each day, with any refusals removed at 0730 h the following day prior to the next day's ration being presented. Peripheral body temperature measurements were taken over two 24-hour periods at hourly intervals 0700 -

2400 h, and two-hourly between 2400 – 0600 h. Core temperature was measured using iButton® temperature logging devices (DS1921H-F5#, accuracy $\pm 1^{\circ}\text{C}$, resolution 0.125°C) placed intravaginally measuring at 10 minute intervals. Vaginal temperatures have been shown to correlate highly with rectal temperatures in previous studies in sheep (George *et al.* 2014) and cattle (Suthar *et al.* 2013) and were considered indicative of core body temperatures for the purposes of this experiment. Placement of continuous monitoring devices rectally was not feasible in this experiment. Peripheral parameters were measured by temperature sensing microchips (Destron Fearing 2009) and recorded using a wand reader (Allflex® RS420) at a range of <20cm.

3.2.4 Design – Experiment 2: Variation in feeding times

The impact of feeding schedule on core body temperature and its rhythmicity was tested by offering the feed ration in feeding schedules of one (FS1), two (FS2), three (FS3) and five (FS5) equal portions per day (data from Experiment 1 was used for FS1). All sheep cycled through all feeding schedules together to prevent distress in the sheep. Core body temperature was measured every 5 minutes over four 24-hour periods with iButtons® secured to a CIDR device intravaginally.

Feed portions and feeding times were varied according to feeding schedules outlined in Table 3.2. At 1700h accumulated daily refusals were weighed and removed.

Table 3.2 Description of feeding schedules

Feeding Schedule	% of ration given at each feed	Feed times
FS1*	100	07:30,
FS2	50	07:30, 13:30.
FS3	33.3	07:30, 11:30, 15:30.
FS5	20	07:30, 09:30, 11:30, 13:30, 15:30.

*FS1 data was collected in Experiment 1

3.2.5 Temperature monitoring devices

A blank (leached) controlled intravaginal drug release device (CIDR®, distributed by Zoetis) was used to secure an iButton® temperature logging device (Model number DS1921H-F5#, accuracy $\pm 1^{\circ}\text{C}$, resolution 0.125°C)(Wolaver and Sharp Jr 2007). The iButton sensors were configured to record vaginal temperature (Tv) readings at intervals of 5 minutes throughout the experimental period. The iButton® sensors were secured to the CIDR using shrink-wrap.

Temperature sensing LifeChip® microchips (Destron Fearing) consisted of a BIO-THERMO® temperature sensor (accuracy $\sim \pm 0.5^{\circ}\text{C}$, resolution $\sim \pm 0.1^{\circ}\text{C}$.) coated with a

BioBond® Anti-Migration Cap (Identipet 2016), which is a porous polypropylene polymer sheath that encourages the accumulation of tissue to hold the microchip in place and prevent migration (Destron Fearing 2009). One microchip was implanted transversely in the sternocleidomastoid muscle (Tn) at a depth which would ensure the microchip was fully embedded in the muscle, and a second was implanted subcutaneously beneath the tail (Tt). An Allflex® RS420 wand reader was used to read the microchips by holding it at a distance of 10-20 cm from the chip site for a few seconds. The animals became habituated to the reading procedure during the initial acclimation period. The peripheral measurement sites were chosen with consideration given to limiting carcass downgrade when implants are removed. Implantation was performed two weeks prior to commencement of the experiment and no inflammatory response to the implants was noted. Freedom of movement of the animals did not appear to be affected following the implantation of the microchip. The microchips were not removed at the conclusion of the experiment and will remain in place for the life of the animal. Ambient temperatures were recorded from iButton® loggers (Model number DS1921G-F5#, accuracy $\pm 1^{\circ}\text{C}$, measures in 0.5°C increments) positioned at points at the northern, southern, western, eastern and central points of the sheep pens which would give a representative ambient temperature for the sheep pens. These loggers recorded temperature measurements at intervals of five minutes throughout the trial period. Outside temperatures during the trial period ranged from 7.6 to 29.5°C , with relative humidity for the region ranging from 61 to 95% (Australian Bureau of Meteorology 2015).

3.2.6 Data and statistical analyses

Due to time constraints during the experiment there were not enough peripheral measurement data to include in this experiment.

As activity associated with feeding may increase body temperatures, data was also analysed with peaks believed to be associated with this feeding activity removed. These peaks were identified visually in the raw data as being associated with feeding activity due to timing and duration. Two sets of data were generated, those with peaks associated with feeding activity removed and those without.

3.2.7 Circadian fitted curve analysis:

The data collected from each animal constituted a 24-hour cycle from Tv, Tn and Tt at hourly intervals for the first 18 hours from 0700, and two-hourly for the remaining 6 hours. Tv data was collected more frequently (at 5 and 10 minute intervals); however, these data were

condensed into hourly measures in the analyses of Experiment 1 as peripheral temperatures were collected manually and less frequently, the condensed data was measures taken at time points closest to the peripheral measures. The same methodology was applied to the measures in Experiment 2.

CircWave software (v 1.4, 2007 R. Hut) was used to produce a Fourier-curve for each data set using harmonic regression, this data produced “goodness of fit” R^2 characteristics for each curve.

Once the Fourier-curve periodogram model was generated for each data set using CircWave v1.4 software (an extension of the cosinor analysis, courtesy of Dr Roelof Hut) (Hut 2007), the fitted curve data was then used to characterise averaged circadian rhythm parameters for each sheep on each measurement day. These rhythmic parameters described: amplitude (the difference between the peak and the mean value of a wave), mesor (a circadian rhythm-adjusted mean), range of oscillation (difference between maximum and minimum observed values) and acrophase (the time at which the peak occurs) (Circadian Rhythm Laboratory 2017).

Once the raw data was transformed through CircWave and circadian parameters characterised, the statistical software R (v 3.3.1, 2016) was used for the remainder of the statistical analyses.

3.2.8 Experiment 1: To compare the difference in means between each measurement site (Tv, Tn and Tt) for CircWave curve characteristics, a two-sided paired t-test was performed across the rhythm characteristics trough, peak, acrophase, range of oscillation, mesor, standard deviation and amplitude.

A repeated measures linear mixed effect interaction model using *lme* (Pinheiro *et al.* 2011) was used to compare the slopes of the Tv circadian rhythm curve at 1 hourly time points between the different peripheral sites. A random effects model was used to assess the interaction of each site and time within each sheepID. In this interaction model, all one hourly interval slopes were joined together in the plot as a circadian curve, allowing the assessment of site measurement differences in the slope of the curve. Time and site were included in the model as fixed effects with sheep ID added as a random effects term to identify within animal variance. The difference in slope for each hourly interval (eg time at x and $x+1$) was assessed individually as many individual linear slopes at time_{*i*} to time_{*i*+60mins} (n=23).

The significance of the difference in slope was compared from random between the Neck vs Tail vs Vaginal treatment for each hourly interval ie $x_1y_1:x_2y_2$, then $x_2y_2:x_3y_3$ for 23 intervals (of 60 minutes each) where x =time, y =temp and x_iy_i =temp at time i . This allowed an hourly (hr to hr) assessment of curve slope difference. Temperature data for predicted fitted values from the circwave function was log transformed to normalise data for these comparisons and Q-Q and residual plots were tested for normality to ensure that assumptions were correct and the most appropriate test used. Some biologically questionable values below 35°C were removed from the data from one sheep. Means were plotted showing range of variation across individuals at hourly time points throughout the day.

3.2.9 Experiment 2: A two-sided paired t-test comparison of the difference between means was performed on the circadian rhythm characteristics to observe the impact of different feeding schedules on the body temperature pattern, and the effect of removing peaks associated with feeding activity on the rhythmicity of vaginal temperature measurements.

One sheep was omitted from Tv readings for FS2, FS3 and FS5 portions of the trial due to difficulty with logger placement, therefore we were able to analyse the core body temperature rhythm for 10 sheep.

Some biologically questionable values below 35°C were removed from the FS1 data from one sheep.

3.3 Results

3.3.1 Experiment 1

3.3.1.1 Curve parameters

Analysis of the CircWave fitted curve for each sheep indicated that R^2 values ranged between 0.424 and 0.995 for T_v , between 0.465 and 0.967 for T_n , and between 0.503 and 0.954 for T_t ; suggesting that the fitted Fourier curves were a reasonable fit of the 6 measurements/hour.

3.3.1.2 Analyses of fitted parameters.

Pairwise analysis of the differences between the fitted vaginal and peripheral circadian curve parameters revealed that there were significant differences ($P < 0.05$) between core and both peripheral sites for all circadian parameters, with the exception of acrophase (Table 3.3). The peripheral sites both measured lower fitted temperatures than T_v ; T_n by an average of -0.846°C (95% CI -3.088 - 1.395), and T_t by an average of -1.715°C (95% CI -3.873 - 0.444), when compared to mean T_v .

While the above results demonstrated that fitted temperature curve parameters from the Fourier transform procedure differed between T_v , T_n and T_t , pattern similarities were observed at time points across the measurement day; with T_v , T_n and T_t exhibiting similar rhythmicity according to the time of day. This pattern was reflected in the repeated measures interaction analyses, in which site and time interactions were analysed. The model analysed these interactions for the fitted data at each time point across a 24-hour period, with results showing a short period of slope dissimilarity between T_v and T_n from 14-16 hours ($P < 0.05$), and a much longer period of dissimilarity between T_v and T_t from 10-21 hours ($P < 0.05$) (Fig. 3.2). Between sheep variation was increased with the peripheral measurements, and variation analysis showed some variation in T_n data and greater variation in T_t data (Fig. 3.3).

Table 3.3 Pairwise comparisons for the mean difference between circadian rhythm parameters of vaginal temperature and peripheral temperature at the neck and the tail. Data are shown as mean $\pm$ s.d., and mean difference (95% confidence interval), n=10 animals.

Site	Vagina	Neck	Tail	Vagina - Neck (95% CI)	Vagina - Tail (95% CI)
Trough	38.61 $\pm$ 0.25	36.51 $\pm$ 1.13	34.52 $\pm$ 1.15	2.10 (1.35, 2.86)****	4.10 (3.25, 4.05)****
Peak	39.57 $\pm$ 0.24	38.08 $\pm$ 0.74	37.11 $\pm$ 0.47	1.49(1.05, 1.92)****	2.49 (2.10, 2.87)****
Acrophase	11.78 $\pm$ 2.17	13.39 $\pm$ 2.29	14.12 $\pm$ 2.50	-1.61 (-2.84, -0.38)*	-2.31 (-3.67, -0.89)**
Range of oscillation	0.96 $\pm$ 0.23	1.57 $\pm$ 0.60	2.59 $\pm$ 1.15	-0.61 (-1.00, -0.23)**	-1.62 (-2.41, -0.83)**
Mesor	39.09 $\pm$ 0.25	37.35 $\pm$ 0.90	35.92 $\pm$ 0.70	1.75 (1.21, 2.29)****	3.19 (2.63, 3.75)****
Standard deviation	0.26 $\pm$ 0.06	0.49 $\pm$ 0.20	0.85 $\pm$ 0.36	-0.23 (-0.37, -0.9) **	-0.58 (-0.84, -0.32)***
Amplitude	0.48 $\pm$ 0.15	0.74 $\pm$ 0.25	1.19 $\pm$ 0.52	-0.26 (-0.41, -0.12)**	-0.71 (-1.05, -0.36)**

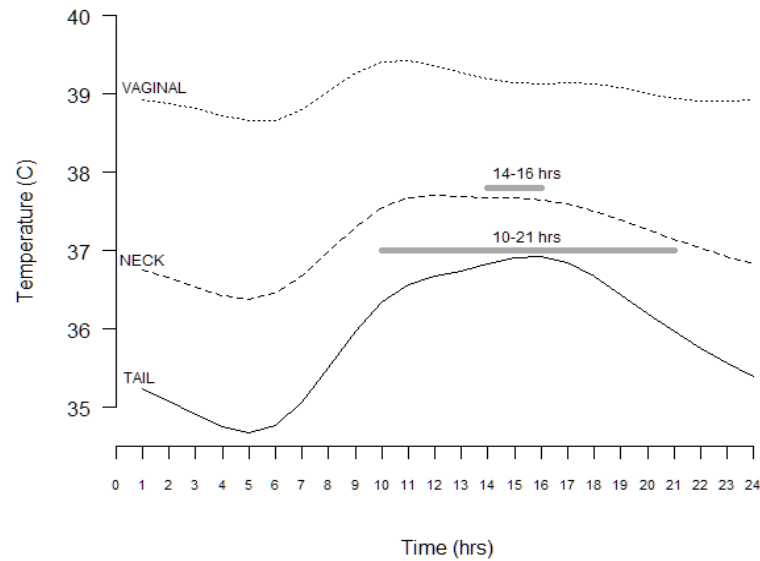


Fig. 3.2. Comparison of the 24 hr circadian rhythms at vaginal, neck and tail sites using hourly time points in n=11 animals. While slopes demonstrate hourly means, the horizontal bars indicate time periods during which peripheral measurement pattern slopes were significantly different from Tv ($P < 0.05$) when adjusted for measurements collected on the same animal.

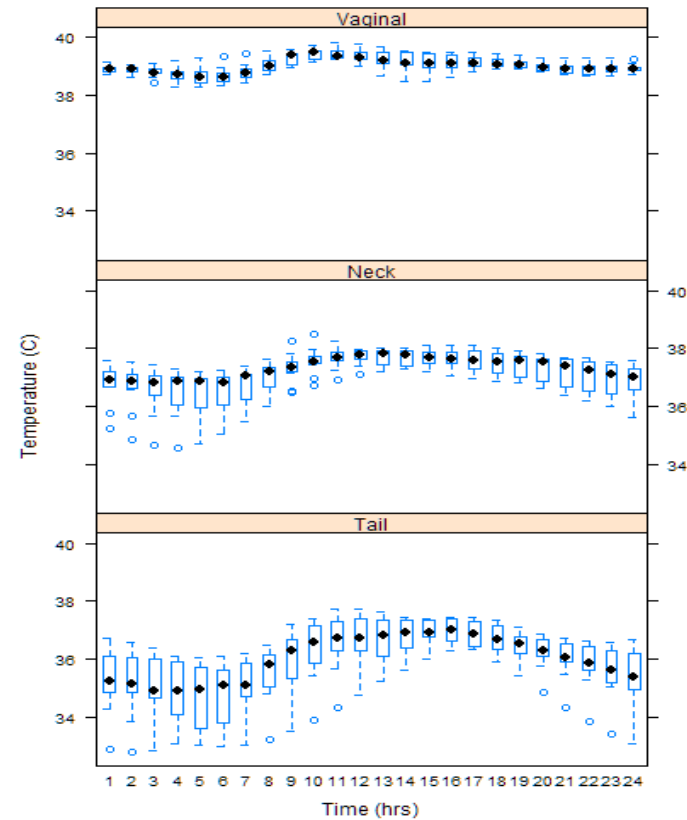


Fig. 3.3 Variation in circadian rhythm across n=11 animals at 1 hourly time points for vaginal, neck and tail. Black dots represent median values for each hourly measurement and single hollow circles represent outlier values (1.5 times above or below the interquartile range).

3.3.2 Experiment 2

Consumption of feed differed across feeding schedules. During feeding schedule 1 the entire ration was consumed within approximately 0.5hr, with increased refusals collected as the frequency of feeding increased for approximately 40% of the animals. The rest of the animals consumed the entire ration throughout the experimental period.

The impact of different feeding schedules on the body temperature pattern was observed, and also the effect of removing peaks associated with feeding activity on the rhythmicity of vaginal temperature measurements. Rhythm parameter means differed significantly across the feeding schedules, with a general trend showing a smoothing of the rhythm as number of feeds per day increased. A similar trend was observed in the comparisons when temperature peaks associated with feeding activity were removed (Table 3.4), the peaks associated with feeding activity did not impact on the overall variation or rhythmicity over the day for feeding schedule 2-5, but were observed to occur over short time periods (< 1hr) and impacted on the rhythm parameters peak, acrophase, range of oscillation, mesor, standard deviation and amplitude during FS1.

Pairwise comparisons were analysed for Tv conducted on each feed regime and each measure, where peaks associated with feeding activity were removed. These demonstrated significant differences between peak, acrophase, range of oscillation, mesor, standard deviation and amplitude between data with feeding peaks and data with feeding peaks removed for data collected during feeding schedule 1 ($P < 0.05$) with less extreme variations when feed peaks were removed. No significant differences were found for the trough parameter in feeding schedule 1, or any of the parameters for feeding schedules 2 through 5 ($P > 0.05$) (Table 3.4).

Table 3.4 Mean ($\pm$ s.d.) values for vaginal temperature (Tv) for the parameter values observed for each feeding schedule, with and without peaks associated with feeding activity.

	Parameters	Schedule 1 mean	Schedule 2 mean	Schedule 3 mean	Schedule 5 mean	Significance of differences between feeding schedules (<i>P</i> -value)
With feed peaks	Trough	38.59 ^b $\pm$ 0.20	39.06 ^a $\pm$ 0.21	39.06 ^a $\pm$ 0.34	38.70 ^b $\pm$ 0.33	*
	Peak	39.70 ^{aby} $\pm$ 0.31	39.90 ^a $\pm$ 0.33	39.56 ^b $\pm$ 0.30	39.54 ^b $\pm$ 0.27	**
	Acrophase	10.46 ^{by} $\pm$ 2.30	14.22 ^a $\pm$ 4.01	16.28 ^a $\pm$ 3.69	14.76 ^a $\pm$ 3.53	*
	Range of oscillation	1.11 ^{ay} $\pm$ 0.26	0.83 ^a $\pm$ 0.25	0.53 ^b $\pm$ 0.13	0.86 ^a $\pm$ 0.31	**
	Mesor	39.00 ^{cy} $\pm$ 0.17	39.49 ^a $\pm$ 0.24	39.30 ^b $\pm$ 0.29	39.12 ^c $\pm$ 0.23	*
	Standard deviation	0.27 ^{ay} $\pm$ 0.06	0.23 ^a $\pm$ 0.08	0.15 ^b $\pm$ 0.06	0.26 ^a $\pm$ 0.11	*
	Amplitude	0.70 ^{ay} $\pm$ 0.26	0.38 ^{by} $\pm$ 0.13	0.28 ^c $\pm$ 0.08	0.42 ^b $\pm$ 0.16	*
Without feed peaks	Trough	38.61 ^b $\pm$ 0.18	39.19 ^a $\pm$ 0.22	39.01 ^a $\pm$ 0.36	38.74 ^b $\pm$ 0.18	*
	Peak	39.41 ^{cz} $\pm$ 0.20	39.84 ^a $\pm$ 0.37	39.71 ^{ab} $\pm$ 0.29	39.52 ^c $\pm$ 0.29	*
	Acrophase	12.75 ^z $\pm$ 3.25	15.42 $\pm$ 4.09	10.94 $\pm$ 8.82	12.67 $\pm$ 6.18	n.s.
	Range of oscillation	0.80 ^z $\pm$ 0.18	0.63 $\pm$ 0.35	0.73 $\pm$ 0.33	0.78 $\pm$ 0.29	n.s.
	Mesor	38.96 ^{dz} $\pm$ 0.15	39.50 ^a $\pm$ 0.20	39.33 ^b $\pm$ 0.28	39.16 ^c $\pm$ 0.24	*
	Standard deviation	0.23 ^{az} $\pm$ 0.07	0.19 ^{ab} $\pm$ 0.11	0.17 ^b $\pm$ 0.05	0.23 ^{ab} $\pm$ 0.08	*
	Amplitude	0.45 ^z $\pm$ 0.10	0.33 $\pm$ 0.24	0.39 $\pm$ 0.20	0.36 $\pm$ 0.12	n.s.
Comparison between means with and without feed peaks		<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	
	Trough	n.s.	n.s.	n.s.	n.s.	
	Peak	*	n.s.	n.s.	n.s.	
	Acrophase	*	n.s.	n.s.	n.s.	
	Range of oscillation	*	n.s.	n.s.	n.s.	
	Mesor	**	n.s.	n.s.	n.s.	
	Standard deviation	*	n.s.	n.s.	n.s.	
	Amplitude	*	n.s.	n.s.	n.s.	

Data are means $\pm$ s.d., n = 10. Within a row, means not sharing a common letter differ (a, b, c, d) (*, $P < 0.05$, **, $P < 0.01$, n.s. not significant), within a column, means not sharing a common letter differ (y, z) (*, $P < 0.05$, **, $P < 0.01$, n.s. not significant).

Temperature peaks or spikes associated with feeding activity had a significant effect on the circadian rhythm of feeding schedule 1, but not for schedules 2, 3 or 5. Feeding schedule caused changes in fluctuations in Tv over time, with the more extreme fluctuations seen in feeding schedules 1 and 2, than in 3 and 5. This pattern is illustrated for Sheep 04 in Fig. 3.4, with similar trends being observed for data both with and without peaks associated with feeding activity.

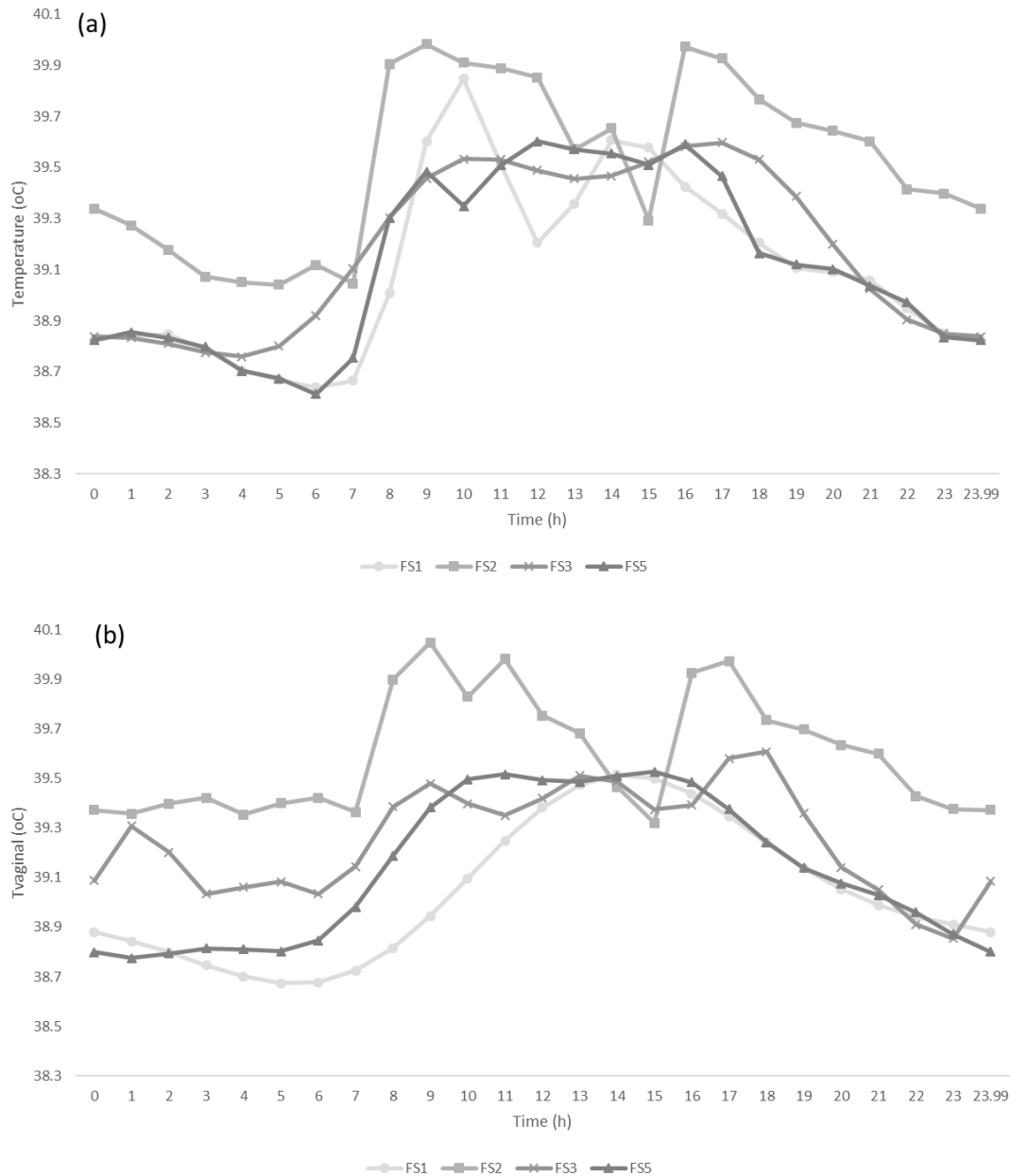


Fig. 3.4 Changes in TVagina (°C) over time for sheep 04 (a) with feed peaks included, and (b) with feed peaks removed. Data is fitted curve values for each regime over 24 hours. FS = feeding schedule, daily ration broken into 1 (FS1), 2 (FS2), 3 (FS3) and 5 (FS5) feeds per day.

3.4 Discussion

The results from Experiment 1 suggest that the ability of peripheral temperature measurements to reflect core temperature may be impacted by several factors relating to both thermophysiology and the effectiveness of the measurement device and placement, particularly with consideration to the skin as a thermoregulatory organ. While there were differences in the parameters usually used to compare body temperature measures, similarities in the rhythmicity found in the analysis of these results suggest that peripheral measures may have utility in the detection of circadian rhythm changes. Many of the findings of studies done on anaesthetised animals may also present differently in conscious animals.

In some previous studies, peripheral temperature measurements have shown some promise as alternative measures of body temperature in sheep (D'Alterio *et al.* 2011) using infrared thermography (IRT) to observe foot temperature and comparing circadian characteristics; piglets (Chung *et al.* 2010) also using IRT looking at correlation between several surface temperature sites and rectal temperature, finding three areas with significant correlation, the abdomen, dorsal area and perianal area; and horses (Ramey *et al.* 2011) using a non-contact infrared thermometer (NCIT) to obtain temperatures from the premaxillary gingiva area which correlated poorly with rectal temperatures. However, the measures from NCIT were able to reliably detect fever; and in rabbits (Chen and White 2006) compared IRT and temperature sensing microchips with rectal temperature, finding that the microchips more closely reflected core temperatures. These previous studies used raw values and rhythm parameters to compare measurement locations. Our work sought to identify any differences in conscious sheep between the peripheral measurement locations chosen and core temperatures; other work has shown no relationship between core measures and peripheral measures in a number of species. Tomasic (1999) used a catheter to measure pulmonary artery temperature (core) and thermistors at rectal, nasal, groin and skin in anaesthetised horses, noting that nasal groin and skin followed a biphasic pattern, and peripheral measures differed depending on the time under anaesthesia but there was no relationship between core measures and peripheral measures. A similar study in anaesthetised sheep by Mansel *et al.* (2008) also found no relationship between core measures and peripheral measures using a thermistor inserted into a catheter in the pulmonary artery to measure core temperature, and thermistor probes to measure rectal temperature and peripheral temperatures at the coronary band and ear base. That study did not investigate circadian rhythm characteristics, or patterns of change. Research in humans (Thomas *et al.* 2004) also failed to find a relationship between core measures and peripheral

measures when comparing rectal temperature with axillary and thoracic skin temperatures. When investigating the circadian rhythms for each, thoracic temperature showed higher variability than axillary skin temperatures, with proximal skin temperature being positively related to rectal temperature and distal skin temperatures showing an inverse relationship with rectal temperatures (Thomas *et al.* 2004). The results from Experiment 1 of the present study showed that peripheral temperature measures at neck and tail sites and their circadian parameters differ from absolute core temperature measurements. Therefore, in order to assess their utility in temperature change detection it was necessary to also evaluate the circadian rhythm parameters and patterns of change as they compare between peripheral and core temperature measures.

Previously, circadian patterns have been observed in sheep body temperatures as measured by both rectal temperature (Mohr and Krzywanek 1995; Piccione *et al.* 2002a; MacLeay *et al.* 2003; Piccione and Caola 2003), and vaginal temperature (Hunsaker *et al.* 1976). Foot temperature measurement taken with infrared thermography (IRT) suggests a daily rhythm (D'Alterio *et al.* 2011), which indicates that peripheral temperature measures may be of use in detecting circadian temperature patterns in sheep. However, the results of the present study found significant differences between the core and peripheral sites for most of the common circadian parameters used in rhythm analyses. Of note regarding the utility of peripheral measures for diagnostic purposes is the pattern analysis performed through a repeated measures linear model; this showed that while absolute temperatures differed significantly from T_v for both T_n and T_t , the pattern of change showed similarities, particularly between T_v and T_n . This results suggests that T_n could be of use in determining a threshold at which a temperature differs from normal at any given time point along the daily oscillation, and may be a useful reflection of patterns of core temperature, and possibly changes of clinical significance. An example of the clinical utility of peripheral measurements can be seen in Ramey *et al.* (2011) in which it was shown that absolute temperatures did not correlate well with core (rectal) temperature measurements; however, the peripheral measures were still considered to be capable of reliable detection of temperatures which were more than “clinically normal” and therefore may be utilised in a diagnostic or health monitoring capability.

T_t measurements varied more than those of T_v and T_n . This variation could be the result of the subcutaneous placement of T_t being closer to the skin surface than T_n (which was located deeper into the muscle tissue) and therefore subject to greater activity due to the role of the skin in thermoregulatory processes (Hales *et al.* 1978; Kuhnen and Jessen 1988). The greater

variation exhibited by Tt may also have resulted from the skin being subject to greater influences of ambient temperature, a phenomenon which is also varying depending on the presence of the insulating properties of wool (Cockrem and Wickham 1960). Additionally, the thickness of the skin at the two sites varies. Skin at wool-growing regions in sheep is known to be thinner than skin on hair growing regions, and this skin thickness impacts on heat transfer at the surface of the skin and the role of the skin in thermoregulation (Lyne and Hollis 1968). The skin overlaying the neck muscle grows wool and the skin on the underside of the tail is largely bare and is consequently thinner. The depth at which the sensor was placed is also considered to have impacted on this variation, with Tn microchip being placed deep into the neck muscle while Tt was placed subcutaneously just under the skin on the underside of the tail. It is also known that adjustment of skin temperature is predominantly centred on the extremities as opposed to the skin on the trunk of the body, and therefore the greatest variation in skin temperature occurs at the extremities (Yousef 1986), and distal regions of the body are rich in arteriovenous anastomoses which regulate blood flow and play a key role in thermoregulation (D'Alterio *et al.* 2011). In consideration of the known data these results suggest that Tn is potentially a more reliable site from which to develop a model of body temperature measurement than Tt.

Variation between temperature measures within and between individuals observed between Tv, Tn and Tt could also be related to the different measurement devices used as the sensitivity of the devices used in this trial varied. While iButtons® have been used to reliably record body temperature measurements in other experiments (Piccione *et al.* 2013b; Wallage 2014), the temperature sensing microchips are more sensitive and this sensitivity may influence the variability of the measurements from the microchips. This sensitivity may be particularly apparent with very small changes in temperature in the surrounding tissue, such as can be observed resulting from factors influencing skin temperature (handling, ambient temperature, and insulation of the skin) (Cockrem and Wickham 1960). Further investigation into these possible sources of variation is warranted, and may be of relevance in determining the applicability of these measures in detection of clinically significant temperature changes.

Once implanted, temperature logging devices such as iButtons® do not require the sheep to be handled for the measurements to be taken; however, the readings cannot be downloaded until the device is removed from the animal. In contrast, temperature sensing microchips can be read with minimal handling with a wand reader or panel reader, and deliver a real-time measurement. There are currently limitations regarding data collection if repeated

measurements are desired; these are thought to be primarily associated with proximity of the reader to the animals and the receptivity of the antenna and reader. Thus, close handling is still required to obtain regular repeated measurements with a wand or panel reader. Additionally, the microchip devices remain in place for the life of the animal; and as a result, the placement of microchips may impact on the carcass value.

There are several external factors influencing body temperature measurements and these need to be accounted for in order to determine the applicability and practicality of a new method or technology for temperature measurement. The comparison of feeding schedules in Experiment 2 showed significant differences for many of the rhythmic parameters across all feeding schedules. These results suggest that frequency of feeding and feed type may have some influence on the rhythmicity of body temperature in sheep and may be related to the circadian rhythms of rumination and metabolism which are known to be influenced by environmental factors. Observed temperatures have been shown to be affected by factors such as feeding rates and times in sheep and cattle (Maloney *et al.* 2013; Nikkhah 2014), shearing (Piccione *et al.* 2002a; Piccione and Caola 2003), season and physiological status (cattle) (Kendall and Webster 2009), the age of the animal (Piccione *et al.* 2002b), and time during the day of measurement (Refinetti and Menaker 1992). The results parallel previous work which investigated the effect of breed, splitting feeds and time of feeding on vaginal body temperatures of hair sheep ewes under tropical conditions, with results showing influences of both breed and time of feeding (Godfrey *et al.* 2013). The variation seen, therefore, in the data with feeding peaks removed is likely to be attributable to the natural rhythmicity of body temperature (Refinetti and Menaker 1992). As a consequence of this variation, it is necessary to interpret temperature data in relation to the time of day and likely baseline temperature at that time point, and with consideration to the timing of feeds and the possible influence of increases in temperature associated with feeding activity.

The effect of temperature peaks/spikes on the rhythm of FS1 suggests that while peaks associated with feeding activity may influence the expression of the circadian rhythm, this effect is only significant for FS1, where most of the food for the day is eaten early in the day, which suggests that adding feeds more frequently, or providing *ad libitum* feed may reduce the effect of feeding activity on the body temperature rhythm. However, the presence or removal of feeding peaks associated with feeding activity does not generally appear to impact on the gross rhythmicity of body temperature, with temperatures showing a normal diurnal pattern of higher temperatures in the early evenings and lower temperatures in the early mornings.

The importance of the careful evaluation of normal circadian rhythms of body temperature is evident in these findings, as the different measurement methods and locations can result in significant variations from normal vaginal body temperature both in circadian rhythmicity and absolute temperatures. This has implications for their use in practical applications as an alternative to core temperature for diagnostic and clinical purposes. Further investigation of these variations and their causes is warranted when utilising peripheral temperature measurements, particularly on their ability, or otherwise, to reflect normal circadian patterns; as well as the exogenous and endogenous influences on thermoregulation at the periphery. Additionally, the influence of feeding activity and feeding regimes encompassing the normal expression of body temperature rhythmicity should be considered when interpreting body temperature data collected at different time points throughout the day.

3.5 Conclusion

Peripheral temperature measurements may have the potential to provide a useful body temperature measurement; however absolute values obtained from peripheral locations differ from those obtained from core (vaginal), probably due to thermoregulatory influences at peripheral locations. The location of the device appears to have some influence on observed temperature, variability of measurement, and rhythmicity, with T_n showing less variation than T_t when compared to T_v. The findings presented do not exclude peripheral measures from use in a clinical utility, but further investigation is needed to determine the usefulness of these measures, particularly with regards to establishing normal parameters for individuals to determine clinically important deviations from same. Additionally, consideration should be given to the most appropriate measurement locations in sheep, given the degree and predictability of the variation shown with the peripheral measurement devices.

Analysing rhythmicity of measurements may assist in the determination of clinically important temperature thresholds pertaining to the use of these devices and locations. Additionally, when analysing circadian patterns of vaginal temperature, consideration should be given to feeding regime and its influence on the expression of daily rhythmicity.

Chapter 4

Daily rhythmicity of body temperature, under normal conditions and during fever challenge in peripheral temperature measurements in sheep.

Abstract.

Core body temperature is a key indicator of animal health and the ability to measure body temperature at peripheral locations may be important in the development of non-invasive and minimally invasive tools for remote temperature measurement in livestock. This study aimed to investigate the similarities between core and two peripheral sites when measuring temperature changes of sheep under a fever challenge. Eleven crossbred ewes were implanted with temperature sensing microchips which were positioned transversely in the sternocleidomastoid (neck) muscle and subcutaneously under the tail. Temperature measurements were taken at 10 minute intervals from the microchips and from an iButton sensor placed intra-vaginally to record core temperature. Circadian core and peripheral temperatures were recorded during baseline periods to establish normal rhythmicity for each individual, and during the challenge period when sheep were exposed to challenges designed to increase core temperature. Significant differences were observed between challenge and baseline cosinor adjusted data for all parameters for T_v , and most for T_n while, for the purposes of animal health monitoring, subcutaneous T_t measurements showed minimal detection of treatment effect ($P < 0.05$).

4.1 Introduction

Detection and treatment of illness and disease in sheep can be delayed in extensive and other low input production systems due to time and labour constraints (Dwyer 2009), and thus, animal health and welfare may be compromised (Lawrence and Appleby 1996; Petherick 2005; Goddard *et al.* 2006). Since many common ailments can be alleviated or prevented through timely treatment and management protocols (Roger 2008), the ability to remotely measure key indicators of animal health would potentially be of benefit in these systems.

Body temperature change is commonly used as an indicator of compromised health in an animal, with rectal temperature measurement used as a common diagnostic tool (Sargison and Scott 2010). Core body temperature can be measured using several methods, including a rectal digital thermometer (Colditz *et al.* 2005), a temperature logger for vaginal temperature measurement (Suthar *et al.* 2013), a thermocouple surgically positioned within the peritoneum (Smith *et al.* 2011), and a pulmonary arterial thermistor inserted via cardiac catheterisation (Mansel *et al.* 2008).

Many of these existing methods of body temperature measurement are invasive and require close handling of the animals, which is known to increase stress levels in sheep (Hemsworth *et al.* 2011). The difficulty in obtaining accurate core temperature measurements and the desire to minimise handling of animals, means that alternative temperature measurement systems and sites e.g. peripheral sites, may be useful in remote detection of illness or disease in sheep (Langer and Fietz 2014).

There are several thermo-physiological states which can be indicative of compromised health in an animal. For example, fever and hyperthermia are two very different thermoregulatory phenomena; the former being a controlled increase in core temperature, and the latter resulting from the inability to lose heat gained as a result of either endogenous or exogenous processes (Hales and Sakurada 1998). Fever is generally defined as a pathological condition involving an abnormal increase in core temperature; however, the extent of this rise can vary and may be defined as being a rise “greater than the mean SD for the species in basal conditions” (Bligh and Johnson 1973). Core body temperature in sheep ranges between 38.5°C and 39.5 °C (Riek *et al.* 1950; Piccione *et al.* 2002b, 2005), with peripheral body temperature measurements varying greatly as described in Chapter 3; however, this variation may be subject to both nychthemeral (24-hour cycle) and seasonal influences, which has been found to account for around 1°C and 0.8°C variation, respectively (Bligh *et al.* 1965). Additionally, the usefulness

of one-off measures may be limited when considering the degree of variation which exists both between and within animals. The variation in body temperature attributed to circadian rhythms has been documented by Refinetti and Menaker (1992) using data from investigations conducted by Bligh *et al.* (1965) and Mohr and Krzywanek (1990) and they indicated that the range of oscillation of body temperature differed, with the former recording 1.0°C variation and the latter recording 0.6°C variation. These findings suggest that the body temperature measured at a given time of day should also take into consideration the range of temperatures which could reasonably be expected in that individual at that time of day. In Chapter 3 of this thesis we found that fitted circadian data and absolute temperature data differed across measurement sites and parameters, with Tn more reflective of Tv (core) temperature variation than Tt. The ability of peripheral measures to detect key circadian rhythm parameters may indicate their utility in a remote animal health monitoring capacity, as compromised health is not only reflected in absolute temperatures, but can also be implicated in diurnal expression of body temperature rhythmicity (D'Alterio *et al.* 2012), and may allow rhythmic parameters and patterns to be used to detect abnormal or clinically significant changes in body temperature. Therefore, the present study aimed to assess the suitability of temperature measurements taken at two peripheral sites using temperature sensing microchips for the detection of clinically significant elevations or decreases in core temperatures, considering daily rhythmicity and individual variation. It was hypothesised that peripheral temperature measures may have utility in the detection of fever and that different peripheral measurement locations would respond differently to core temperature change.

4.2 Materials and methods

4.2.1 Overview

An experiment was undertaken to investigate body temperature changes at core (vaginal) and peripheral measurement sites (neck muscle and subcutaneously under the tail) during a fever challenge. Animals were allowed an acclimation period prior to the experiment to become used to the facilities and handling procedures, and were given a recovery period following the experiment in order to allow the animals to rest and recover before being returned to their familiar paddock. Temperature measurements were taken over a 48-hour period (experimental period), 24 hours prior to the challenge and 24 hours following the challenge. All procedures were approved by the University of New England Animal Ethics Committee, authority number AEC15-096. The care and use of the animals was in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2013).

4.2.2 Animals

Eleven crossbred ewes (six tooth, tail docked as lambs with a short tail left, 10 experimental animals and one spare) were introduced into the animal house 16 days prior to the commencement of treatments and were habituated to handling once a day. The sheep were housed throughout this acclimation period in groups of five or six sheep, and then were introduced into the individual pens two days prior to commencement of the experimental period. The animals were returned to group pens during the recovery period. The animal holding facility was well ventilated with wooden slatted floors, and the pens were swept daily. Mean live weight of the sheep was 61 ± 6.5 kg (mean $\pm$ s.d.), individual sheep weights were used to calculate lipopolysaccharide (LPS) dose.

Sheep were fed each day at approximately 8 am throughout the experiment. Rations were consumed prior to the challenges commencing at 10am and no further feed was given during the challenge time. The sheep were fed a blend of oaten, lucerne and wheaten chaff at maintenance levels (9-12MJ ME/sheep.day) for the whole of the experiment. The sheep were shorn just prior to entering the animal house, had been treated for internal and external parasites, and were in good health, with good body condition and free from disease.

During data recording periods, the sheep were individually penned (2 x 2 m) so that they could not dislodge the measurement devices; companion animals were always in visual and auditory contact.

The experiment was conducted in November 2015, and daily temperatures ranged between 5.6 and 29.2°C, with relative humidity for the region ranging from 25 to 89% (Australian Bureau of Meteorology 2015).

4.2.3 Temperature measurement devices

A leached blank controlled intravaginal drug release device (CIDR, distributed by Zoetis) was used to secure two temperature measurement devices that could record core body temperature changes: an iButton® (Tv) temperature logging device model number DS1921H-F5# (accuracy $\pm 1^{\circ}\text{C}$, resolution 0.125°C , (Wolaver and Sharp Jr 2007). The iButton® sensors (Thermodata) were configured to record temperature readings at intervals of 10 minutes during the baseline and challenge periods. The Tv sensors were secured to the CIDR using shrink-wrap and calibrations were checked against a spirit thermometer.

Temperature sensing LifeChip microchips (Destron Fearing) were implanted at two sites in each of the 11 animals. These microchips consisted of a BIO-THERMO® temperature sensor (resolution $\sim \pm 0.1^{\circ}\text{C}$ and accuracy of $\sim \pm 0.5^{\circ}\text{C}$.) coated with a BioBond® Anti-Migration Cap (Identipet 2016), which is a porous polypropylene polymer sheath that encourages the accumulation of tissue to hold the microchip in place and prevent migration. An RS420 Green Stick Reader (Allflex) wand was used to read temperature measurements from the microchips at a distance of $<20\text{cm}$. Animals were habituated to the proximity of handlers and the reader over the acclimation period, readings were taken as quickly as possible to minimise the stress on the animals. One microchip was implanted in the sternocleidomastoid muscle (Tn) and a second was implanted subcutaneously beneath the tail (Tt). The peripheral measurement sites were chosen with consideration given to limiting carcass downgrade when implants are removed. Implantation was performed nine days prior to commencement of the experiment and no inflammatory response to the implants was noted. Freedom of movement of the animals did not appear to be affected following the implantation of the microchip. The microchips were not removed at the conclusion of the experiment and will remain in place for the life of the animal.

Ambient temperatures (Ta) were recorded using iButton temperature loggers (model number DS1921G-F5# accuracy $\pm 1^{\circ}\text{C}$, measures in 0.5°C increments) distributed across the sheep housing area to be representative of the temperature in the sheep pen area. These loggers recorded temperature measurements at intervals of every 10 minutes for Experiment 1 and every 5 minutes for Experiment 2. For the area in which the experiments were held, and over

the period of both experiments, the environmental temperatures ranged between 5.6 and 29.2°C, with relative humidity between 25 and 89% (Australian Bureau of Meteorology 2015).

4.2.4 Experimental design.

Baseline temperature measurements were taken for 24 hours prior to the commencement of the challenge. A total of 10 sheep were monitored over a 48-h period for the experiment, with a spare available in case a substitution was necessary. In the first 24 h of each treatment period, baseline temperatures of individual animals were recorded using the three measurement devices, and in the second 24 h period, temperature responses to the fever temperature challenge was recorded using the three measurement devices.

4.2.5 Baseline measures

During the baseline measurement period for each experiment, T_n and T_t were measured at intervals of every 1hr from 0900–0100, then every 2 hr from 0100–0700, then every 1hr from 0700–0900, while T_v measurements were recorded at 10 minute intervals.

4.2.6 Fever challenge

The twenty-four hours of baseline measurements were taken during which no injections were given but handling to fit measurement devices was performed prior to measurement commencement. These measures were followed by 24 hours of challenge period measurements. In this experiment, a lipopolysaccharide (LPS) derived from the bacterium *Salmonella abortus equi* (Sigma Aldrich) was dissolved in saline solution administered intravenously at the beginning of the experimental period at a dose rate of 0.03 µg/kg which was likely to induce fever and increase body temperature for a period of approximately 3-4 hours with no lasting negative effects (Smith *et al.* 2011; George *et al.* 2014). Baseline measures were performed without injection of saline however the animals were subject to handling procedures at the beginning of the measurement period.

During the challenge period, T_n and T_t were measured from 1000 (approximate commencement time of challenge when LPS was administered) to 1500 at intervals of 10 mins, every 30 mins between 1500–2100, every 1hr between 2100 –0100, every 2 hr between 0100–0700 and then every 1hr from 0700–0900. T_v measurements were recorded continuously. On completion of the experimental period (48 hours), the CIDRs were removed and the sheep were given a five-day recovery period during which they were monitored prior to being returned to the paddock.

4.2.7 Data and Statistical analyses

Data sets were divided into time periods with data time points used at 10, 30 and 60 minutes for the LPS challenge to compare the accuracy of measurement devices in detection of core temperature under thermal challenge. Time point measures were chosen rather than averages to gain more accurate comparisons across the time points and measurement sites.

4.2.8 Circadian fitted curve analysis:

The baseline data collected for each animal constituted a 24-hour cycle from the neck, tail and vagina sites (Tn, Tt, and Tv) at hourly intervals for the first 18 hours from 7am, and two-hourly for the remaining 6 hours. Tv data was collected more frequently (at 5 and 10 minute intervals for the first 3 hours following fever and cold challenge) however much of this data was not used in the analyses as peripheral temperatures were collected manually and less frequently, therefore only Tv measures which matched the time points for the Tn and Tt measures were analysed.

A Fourier-curve periodogram model was generated for each data set using CircWave v1.4 software (an extension of the cosinor analysis, courtesy of Dr Roelof Hut) (Hut 2007). The fitted curve data was then used to characterise circadian rhythm parameters for each measurement day. These rhythmic parameters described for each fitted curve: amplitude (the difference between the peak and the mean value of a wave), mesor (a circadian rhythm-adjusted mean), range of oscillation (difference between maximum and minimum observed values) and acrophase (the time at which the peak occurs) (Circadian Rhythm Laboratory 2017).

4.2.9 Statistical analyses

Statistical analyses were performed using R software (R Core Team 2016). A paired t-test comparison of the difference in means was performed between each measurement location and its corresponding baseline measurements (Tv, Tn and Tt), on the circadian rhythm characteristics of the fitted curves to observe the effect of the challenge treatments on core and peripheral body temperature rhythms.

A repeated measures linear mixed effect interaction model using *lme* (Pinheiro *et al.* 2011) was used to analyse the baseline and challenge measures. A random effects model was used to assess the interaction of challenge (for each site) and time within each sheepID. In this interaction model, all one hourly interval slopes were joined together in the plot as a circadian curve, allowing the assessment of treatment differences in the slope of the curve,

The difference in slope for each hourly interval (eg time at x and $x+1$) was assessed individually as many individual linear slopes at time $_i$ to time $_{i+60mins}$ ($n=23$).

The significance of the difference in slope was compared from random between the Baseline vs LPS treatment for each hourly interval ie $x_1y_1:x_2y_2$, then $x_2y_2:x_3y_3$ for 23 intervals (of 60 minutes each) where x =time, y =temp and x_iy_i =temp at time i . This allowed an hourly (hr to hr) assessment of curve slope difference. Temperature data was log transformed to normalise data for these comparisons and Q-Q and residual plots were tested for normality to ensure that assumptions were correct and the most appropriate test used. Some biologically questionable values below 35°C were removed from the data from one sheep across both baseline and challenge data sets. These values were omitted from the analysis and fitted wave analysis performed on the remaining data which was then used in further analyses. Means were plotted showing range of variation across individuals at hourly time points throughout the day.

4.3 Results

4.3.1 Ambient temperatures

Mean ambient temperatures for the pen area over the experimental periods are detailed in Table 4., and ranged from minimums of approximately 16°C to maximums of 26°C. Ambient temperatures were within thermoneutral range between 16°C and 24°C (Alhidary *et al.* 2012) for most the trial period.

Table 4.1 Mean ambient temperature (°C) in animal house as measured by iButton temperature loggers during baseline, lipopolysaccharide (LPS) and cold challenge periods.

	Baseline Day		Challenge Day	
	Minimum (°C)	Maximum (°C)	Minimum (°C)	Maximum (°C)
LPS	16.85	22.24	19.69	25.69

4.3.2 Baseline measurements

For the animals used in this trial, the mean normal T_v range was between 38.5 °C and 39.5 °C. Mean baseline temperature measurements taken from the same animals in an earlier experiment (Chapter 3) ranged between 38.9 °C and 39.6 °C, with small spikes occurring at feeding times. Mean temperatures recorded over baseline measurement days by T_n , T_t and T_v were 37.7 °C, 36.9 °C and 39.3 °C respectively, and all showed mean fluctuations of 0.75°C, although mean T_n and T_t ranged below T_v in all measures. The mean difference between the measures ranged from 1.5°C between vaginal and neck temperatures (Table 4.), and 2.4°C between T_v and T_t (Table 4.). Thus, a T_v value of 40.0°C could be equated to 38.4 °C for T_n and 37.5 °C for T_t . During the lipopolysaccharide (LPS) fever challenge, mean T_v peaked around 41.0 °C.

4.3.3 Neck microchips

Significant differences between baseline and challenge rhythms were found for T_v measurements across all parameters ($P < 0.05$). Significant differences between baseline and challenge curves were found for peak, range of oscillation, mesor, standard deviation and amplitude for T_n ($P < 0.05$) (

Table 4.)

4.3.4 Tail microchips

Challenge measurement curve parameters for Tt showed significant differences from the baseline temperature patterns for the rhythm characteristics of peak, mesor and amplitude ($P < 0.05$)

Table 4.).

4.3.5 Repeated measures analysis

The CircWave program generated R^2 and P -values for goodness of fit for each data set from which a fitted curve was generated. The R^2 values obtained were averaged across all data sets and the mean R^2 of 0.74 was determined. All P -values obtained for individual goodness of fit for each of the fitted curves generated were <0.05 . The fitted curve function was applied to 24 hours of data beginning from 0000 on the day of measurement for both baseline and challenge days, as a result it is important to note that the short-lived nature of the LPS treatment means that only 3-4 hours of data collected on the challenge day is reflective of a pyrexia state.

Repeated measures analyses were performed on the fitted curve data for each measurement type between baseline and challenge measures. T_v showed the least variation across individuals and time points, with T_n showing some variation and T_t showing greatest variation (Fig. 4.1). Significant differences were found between baseline and fever challenge measures from the time periods 9-10am, and 12-4pm for T_v , 9-10am and 12 – 3pm for T_n , and 10am-12pm for T_t ($P < 0.05$) (Fig. 4.2).

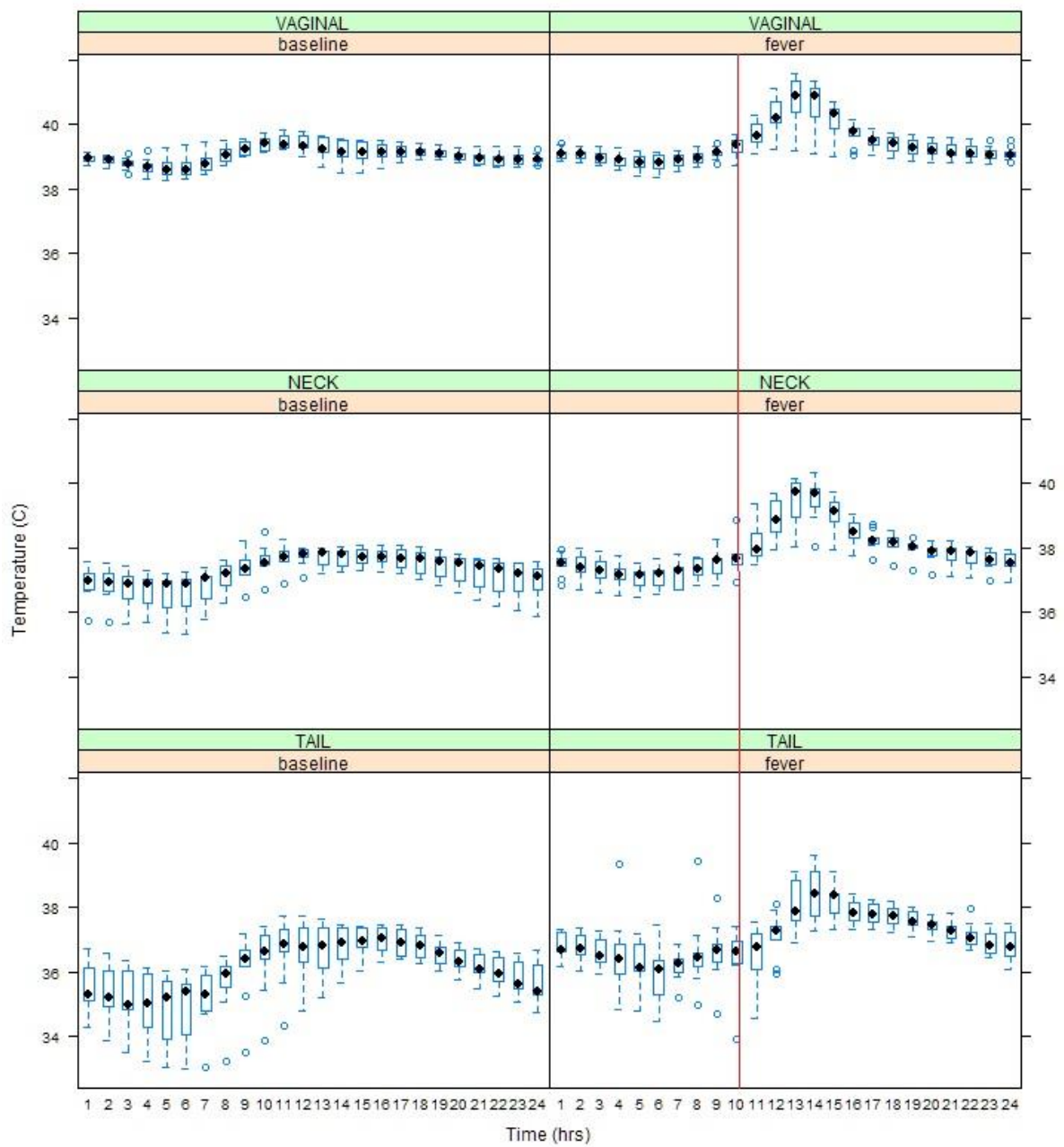


Fig. 4.1 Variation from mean analysis for all sheep comparing baseline and challenge (fever) for each animal at each time point. I, Tv (vaginal); N, Tn (neck); T, Tt (tail). Red line denotes approximate commencement of challenge.

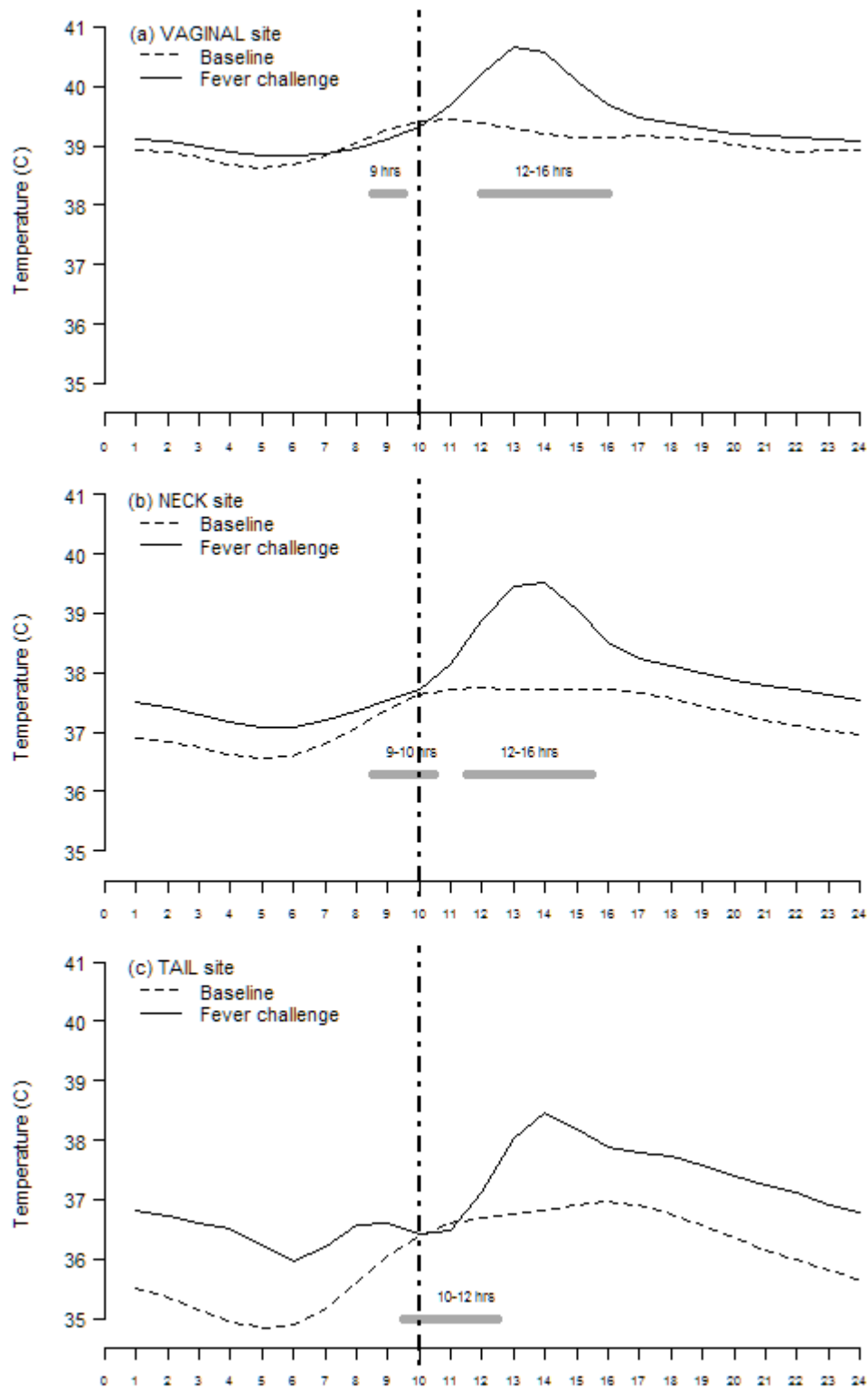


Fig. 4.2 Comparison of baseline and fever response circadian rhythms at the (a) Vagina (Tv), (b) Neck (Tn) and (c) Tail (Tt) sites using hourly time points in n=11 animals. Note that while the curves demonstrate hourly means, the grey bars indicate time periods during which fever challenge temperature patterns were significantly different from baseline ($P < 0.05$) when adjusted for measurements collected on the same animal. Time of challenge for fever response animals was at 10am (dotted vertical line)

Table 4.2 Pairwise comparisons for the mean differences between baseline and challenge fitted curve parameters for each measurement location: Tv, Tn and Tt.

Tv	Baseline	Challenge	Significance of challenge
	mean $\pm$ s.d.	mean $\pm$ s.d.	P
Trough	38.60 ^a $\pm$ 0.23	38.79 ^b $\pm$ 0.25	*
Peak	39.47 ^a $\pm$ 0.20	40.78 ^b $\pm$ 0.78	***
Acrophase	11.84 $\pm$ 1.98	12.81 $\pm$ 1.22	n.s.
Range of oscillation	0.87 ^a $\pm$ 0.27	1.99 ^b $\pm$ 0.65	***
Mesor	39.03 ^a $\pm$ 0.17	39.37 ^b $\pm$ 0.26	***
Standard deviation	0.25 ^a $\pm$ 0.07	0.55 ^b $\pm$ 0.19	**
Amplitude	0.44 ^a $\pm$ 0.17	1.41 ^b $\pm$ 0.57	***
Tn	Baseline	Challenge	
	mean $\pm$ s.d.	mean $\pm$ s.d.	P
Trough	36.72 $\pm$ 0.95	36.93 $\pm$ 0.30	n.s.
Peak	38.03 ^a $\pm$ 0.63	39.60 ^b $\pm$ 0.72	**
Acrophase	13.16 $\pm$ 2.27	13.484 $\pm$ 0.29	n.s.
Range of oscillation	1.32 ^a $\pm$ 0.49	2.67 ^b $\pm$ 0.56	***
Mesor	37.20 ^a $\pm$ 0.40	37.91 ^b $\pm$ 0.33	***
Standard deviation	0.44 ^a $\pm$ 0.14	0.74 ^b $\pm$ 0.16	**
Amplitude	0.63 ^a $\pm$ 0.22	1.70 ^b $\pm$ 0.47	***
Tt	Baseline	Challenge	
	mean $\pm$ s.d.	mean $\pm$ s.d.	P
Trough	34.79 $\pm$ 1.10	35.75 $\pm$ 0.81	n.s.
Peak	37.15 ^a $\pm$ 0.51	38.76 ^b $\pm$ 0.72	***
Acrophase	14.36 $\pm$ 2.47	13.48 $\pm$ 2.14	n.s.
Range of oscillation	2.36 $\pm$ 1.05	3.01 $\pm$ 1.23	n.s.
Mesor	36.03 ^a $\pm$ 0.59	37.06 ^b $\pm$ 0.40	***
Standard deviation	0.79 $\pm$ 0.30	0.83 $\pm$ 0.23	n.s.
Amplitude	1.07 ^a $\pm$ 0.42	1.53 ^b $\pm$ 0.55	*

Data are means $\pm$ s.d., n = 10. Within a row, means not sharing a common letter differ (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; n.s., not significant.)

4.4 Discussion

The present study found that measurements obtained by a temperature sensing microchip placed in the neck muscle may be used to determine the presence of fever by comparing the pattern observed during the fever measurement period with the pattern observed during the baseline measurement period. It was found that LPS challenge influenced core temperature variation within and between animals, and that this influence was also observed in peripheral measurements at the neck muscle site, however minimal influence was observed at the tail site. Circadian rhythm fitted curves generally agreed that T_n was superior to T_t across most of the curve parameters given that there was less variability seen, suggesting that the neck site would be a preferred site for temperature sensing microchip implantation.

Variation in body temperature under thermoneutral conditions was compared and changes observed when ewes were under LPS challenge. The determination of a treatment difference may be ascertained by adding a known amplitude, e.g. 0.4-1.5 °C, to known baseline temperatures, which may be indicative of a fever response in the animal, such as that proposed by Lohse *et al.* (2010) in pigs, who based this on a linear relationship between measures from a rectal thermometer and those from a microchip transponder located by the ear base. Fever temperatures during the trial showed mean maximum temperatures >1.4 °C above mean minimum temperatures across all measurement devices, therefore this increase could be used as a guide when calculating fever alert thresholds. Normal rectal temperature for sheep ranges from 38.3-39.9 °C (Fielder 2017). Baseline temperatures monitored throughout the experimental period are outlined in Chapter 3, and daily rhythmicity was observed, with temperatures dropping early in the morning and rising in the early afternoon, which is consistent with normal diurnal patterns in sheep (Refinetti and Menaker 1992; Saidu *et al.* 2012; Piccione *et al.* 2013a). Slight curve variations between baseline and challenge prior to treatment may be indicative of activities occurring at these time points, feeding prior to 10:00am on baseline measurement days may disrupt the normal temperature measurements at this time, and additional handling on challenge days may also have contributed to this slight variation over the short period prior to treatment commencement. It was considered that a comparison of absolute values was not appropriate for this analysis as absolute values may differ according to many factors such as ambient temperature and activity at any given time point. However, comparing rate of change (slope) over the course of the day shows a difference in the circadian pattern which is more likely to indicate a fever response or compromised thermoregulatory processes than absolute temperature values. This also assists in the

determination of pathological changes which takes into account the effect of the circadian rhythmicity across the day as opposed to comparing time points in isolation which show differences which could be attributable to either fever or a normal change in temperature at a given time of day.

While the neck and tail absolute measurements differ from vaginal (core) temperature measurements, the baseline measures and the treatment response measures followed the diurnal pattern, with the tail and neck measurements differing from core temperature by -1.53°C and -2.47°C , and the tail temperatures showing greater variability which is reflected in the pattern of change and may be attributable to the influence of ambient temperatures and thermoregulatory processes. To determine a baseline measure against which to compare a challenge temperature, it is necessary to match the measurement to the time of day, so as to get an accurate point of difference against the baseline measure. The circadian parameters describe the characteristics of the rhythm, and the significant differences between the parameters exhibited in baseline measures and those exhibited in treatment measures may indicate disease. For example: mean peak may be indicative of a feverish temperature when compared to the normal peak, a change in the mesor indicates a shift in the mean body temperature, the range of oscillation indicates the degree of temperature change over a 24-hour period, and the acrophase indicates at which time the peak occurred. A significantly different acrophase may be useful in the detection of acute fever periods which are outside of the normal peak temperature times, and while our results showed no significant difference in the challenge acrophase compared to that of the baseline, this may have simply been due to the timing of the challenge coinciding with the timing of the peak under normal baseline conditions.

However, it may be difficult to determine a relationship between peripheral and core temperatures using mean temperature data alone, the pattern of change over time could be considerably more useful given the impact of the circadian pattern on body temperature measurements. Tail measures showed a brief period of dissimilarity from 10-12 hours, however the data did not show a response which might reasonably be expected from a fever state, with a decrease in temperature pattern over this period. It is likely, however that the decrease in temperature may have corresponded with stress associated with handling at that time, this temperature decrease may have been due to the activation of the sympathetic nervous system, resulting in vasoconstriction and decreased blood flow at the skin (Stewart *et al.* 2008a). This result demonstrates that influences on temperature measurements at the skin level impact on rhythmic pattern expression, and may explain the increased variability observed in the Tt

measures. The presence of a rhythm does not indicate that disease is not present, as rhythmicity can be maintained while the circadian parameter values may change between baseline and disease states.

This study confirms the presence of a diurnal rhythm in core temperatures in sheep at baseline and under thermal challenge, which corresponds with previous work examining core body temperature rhythms which have been observed in sheep (Refinetti and Menaker 1992) and temperature rhythm detection in peripheral measurement locations (D'Alterio *et al.* 2012).

Our results showed daily rhythmic patterns consistent with circadian rhythmicity for both baseline and fever states, only showing differences in the pattern of change, and this may impact on the usefulness of the data in a clinical or chronic situation where measurements may not be frequent or numerous enough to establish differences in patterns of change and rhythmicity. In some disease states, or when fever is prolonged, the circadian rhythm can be severely disrupted or eliminated (Refinetti and Menaker 1992), and in very young animals, the rhythmicity may not be mature (Piccione *et al.* 2002b) and this is likely to impact on measurement and detection of disease. A possible explanation for the rhythmicity observed in our results may be that the fever response generated by the lipopolysaccharide at the given dosage was not strong or long-lived enough to impact significantly on the observed daily rhythmicity of the temperature measurements, given the timing of the administration of the lipopolysaccharide and the duration of the effect (3-4 hours) it is likely the peak coincided with the normal daily peak and was therefore not seen to impact on the diurnal pattern. However, this method of inducing fever is one which has been utilised in the past with success depending on dose rates, and it may be possible to lengthen the duration of the effect with a continuous or repeated infusion if the impact on daily rhythmicity is to be observed.

When developing an alert system any thresholds should be based on baseline measurements for the individual animal and adjusted for each measurement location rather than utilising an absolute temperature threshold or mean temperature. Work conducted by Tomasic and Nann (1999) utilised a calculation of rate of temperature change in determining similarity between core and peripheral measures, however this study did not compare baseline with temperature change under fever conditions, and it is possible that a rate of temperature change analysis may produce misleading results in subcutaneous measures due to the variability observed here.

Ambient temperatures were not considered for the t-test comparisons, as it was considered that most the experiment was conducted under thermoneutral conditions of between 15-25°C

ambient temperature (Alhidary *et al.* 2012). The effect of ambient temperature was examined (results not shown) and found to be minimal, with any effect being on Tt. This is likely due to the difference in skin thickness at the site, and depth of microchip placement, as well as sympathetically mediated responses at the skin level. This finding is consistent with the findings of Cockrem and Wickham (1960), which showed that ambient temperature has a significant effect on cutaneous temperatures in sheep in addition to other factors such as handling and wool growth. The thermoregulatory processes are impacted by, and impact on, the loss or retention of body heat through blood vessels close to the skin surface, therefore it is expected that ambient temperature will impact on the amount of heat lost or retained from the skin (MacFarlane 1964). Blood flow to the muscle is less subject to these thermoregulatory processes, and thus, is likely to be less influenced by the effect of ambient temperatures. Additionally, the importance of retention of muscle function means that any excess heat will be diverted to the skin and be lost to avoid impaired functionality and decreased contractile properties, effects which may result from increased temperature within the muscle (Locke and Celotti 2014). It is likely that the peripheral site chosen underneath the tail is sensitive to blood flow changes associated with thermoregulatory processes, and the ability to detect the resultant temperature change may be more reflective of core temperature measurements than that of the intramuscular site. However, due to influences of ambient temperature, these results must be considered in the context of a relatively temperate ambient environment, and it is possible that warmer or colder environments may have a different effect on the ability of the tail microchips to detect temperature changes of clinical significance. It is also possible that the restriction of movement necessary in this experiment and the increased handling during treatment and measurement periods, which were difficult to factor into the analyses, may have had some influence on the thermal response of the animal, and these influences would not be present under normal extensive production conditions (Bligh *et al.* 1965).

Due to the short-lived nature of the LPS challenge (3-4 hours), it was not possible to examine the effect of sustained fever over the entire 24 hour period, however in order to evaluate the effect of the challenge period on the circadian rhythmicity and parameters it was necessary to utilise 24 hours of data, which would include the fever period on the challenge day stimulated by the administration of the LPS. Further work could explore the effect of a longer period of fever or challenge.

It was determined that the analysis used to analyse pattern differences was the most appropriate for the investigation of the rate of change of temperature over time. Utilising alternative

analyses, such as area under the curve, may show a change in temperature however incremental changes do not take into account the rate of change and how it may differ between a pattern that could be considered “normal” for that individual, and an abnormal pattern of temperature change which may indicate a disruption to this pattern, and this in turn may indicate fever or some other change not explained by normal temperature change according to circadian rhythm for that individual.

Consideration given when designing future experiments should include longer measurement periods for baseline temperatures, with more regular measurements taken, in order to more readily determine the normal body temperature range across a number of different ambient temperatures and environmental conditions. A controlled climate facility may improve the ability to assess the performance of the peripheral sites across a wider range of ambient temperatures, controlled to remove daily variability.

Ultimately, it is useful to view these findings in the context of the suitability of the measurement locations for detection of core temperatures and core temperature changes, and for investigating the applicability of these peripheral temperature measurements in the detection of body temperature changes of clinical significance in sheep.

The evaluation of the application of this technology in an extensive production system, therefore, requires further investigation to look at the function of the microchips under varying environmental conditions in terms of their ability to reflect core temperature and detect significant temperature changes in general, as well as under varying thermal and health challenges within the animal. Additionally, with regards to the detection of clinical disease, while many diseases show general diagnostic characteristics, including temperature, they may present differently in individual animals, and so the determination of diagnostic thresholds may require further investigation under field conditions.

4.5 Conclusion

The present study has demonstrated that it may be possible that a temperature sensing microchip, placed transversely into the neck (sternocleidomastoid) muscle, could provide a means of reliably detecting core temperature and rhythm changes in mature sheep. A temperature sensing microchip placed subcutaneously under the tail may also detect temperature changes similar to core temperature; however, measures from this site are more variable and more subject to fluctuations in ambient temperature. Additionally, absolute temperatures differ from core, therefore patterns and baseline temperatures should be established using the alternative methods in order to provide a point of reference for each individual animal. Further research to test this application under field conditions, including under varying ambient temperatures and environmental conditions, is warranted.

Chapter 5

Investigating the use of Infrared Thermography in the detection of skin inflammation in sheep at varying fleece lengths.

Abstract

Infrared thermography (IRT) has potential for use in remote monitoring of extensively raised sheep. An initial investigation and then a main experiment were conducted to investigate the potential for infrared thermography to detect skin inflammation in sheep, particularly with regards to inflammation associated with fly strike. The initial validation observations analysed IRT images taken of fly struck sheep to investigate the applicability of IRT in the detection of inflammation associated with fly strike. The main experiment comprised analysis of repeated IRT measurements taken three times daily on 30 sheep over a period of ten days. These measurements were taken to detect an inflammatory response generated by an intradermal administration of a commonly used vaccine formulation, Covexin10®. Patches were shorn into the fleece of each animal at three wool lengths: short (1-2mm), medium (5-10mm) and long (15-20mm). An initial subcutaneous vaccination was administered at 1mL/sheep to prime the immune response, then 0.1mL was administered intradermally into the injection region of the test patches at day 0 and day 7. The validation investigation showed a temperature differential between struck and control areas of +2 to -4°C, likely due to the effect of moisture at the skin surface. In the main experiment, significant differences were found between control and injection sites for the short wool length across measurement days 1-3, 6, 8 and 10, while significant differences were found between injection and control sites across measurement days 8, 9 and 10 for medium wool length and days 2, 3 and 5 for long wool lengths. Variation was lower across the measurements for short wool compared to medium and long wool. These findings suggest that IRT may have application in the detection of skin inflammation in sheep at short wool lengths, and may have limited application at longer wool lengths.

5.1 Introduction

Infrared thermography (IRT) has potential for use in remote monitoring of animal health in extensively raised sheep. Avenues of investigation which are currently being explored predominantly focus on its potential use to measure core body temperature, with George *et al.* (2014) finding a moderate correlation between vaginal temperatures (as measured by digital thermometer) and eye temperature as measured by infrared thermography. Eye temperature has been investigated for its usefulness as an indicator of stress (Stewart *et al.* 2007) and in detection of fever responses (Godfrey *et al.* ; de Diego *et al.* 2013). However such use is limited to clinical detection of fever, and may not be applicable to infections which present as subclinical in the first instance, and localised infections such as fly strike. Infrared thermography may be a useful tool in the detection of skin inflammation, and has been shown to be effective in some animals (Calkosinski *et al.* 2015). However, detection of thermal differentials in the skin is problematic in sheep due to the insulating properties of fleece, and while localised inflammation in sheep has been detected by IRT, this work was done on udder temperatures and therefore did not assess the impact of wool on the surface temperature measurements (Martins *et al.* 2013). Another advantage of IRT is its ability to detect temperatures non-invasively, potentially reducing stress associated with handling which is normally required to detect illness and injury (Stewart *et al.* 2008b).

Fly strike (cutaneous myiasis) is a common health problem in sheep in which skin damage caused by blowfly larvae abrading the skin (Sandeman *et al.* 1987) can result in infection and inflammation in areas where blowfly larvae live and feed on the animal. This damage triggers an inflammatory response at the skin (Bowles *et al.* 1992). This disease is estimated to cost the Australian sheep industry an \$280 million annually (Limited and Australia 2011). Early stages of strike can be difficult to detect and detection often requires mustering and close handling (Cramp *et al.* 2009). IRT has been shown to detect changes in interdigital temperature associated with foot lesions (Talukder *et al.* 2015) and it is therefore reasonable to suspect that it may be useful in the detection of other sources of inflammation. Inflammation can occur at vaccination injection sites (Irish Medicines Board 2009), as well as in response to endotoxins introduced through bacterial infections (Harden *et al.* 2014). Infrared thermography has been shown to detect distinct areas of inflammation in rats (Poljak-Blazi *et al.* 2009; Calkosinski *et al.* 2015) and cattle (Montanholi 2015), therefore it is possible that IRT may be used to detect inflammation resulting from a variety of causes. However, literature regarding the thermal

mechanisms of inflammation is scarce, and simply attributes heat associated with inflammation to increased blood flow to the inflamed area, and therefore information regarding the strength and duration of a heat signal resulting from inflammation is not known. The possibility of remote detection of inflammation associated with blowfly strike presents a unique opportunity for improved detection and management of fly strike in extensive sheep production systems.

Due to the insulating properties of wool however, it is likely that a fleece length greater than 2mm may significantly impact on the ability of a thermal image to reliably identify areas of inflammation of the skin. It was hypothesised that IRT measurements on sheep with vaccination-related inflammation and at varying wool lengths may have application in the detection of associated skin inflammation.

5.2 Materials and Methods:

5.2.1 Overview

An initial validation observation was performed at the University of New England, Armidale, Australia, collecting infrared images of sheep with skin patches affected by fly strike. This investigation was undertaken to determine whether there were differences between fly struck animals and non-struck animals which could be observed with IRT. Sheep were brought in to the yards for routine husbandry management and were identified as struck and non-struck. Infrared thermographic images were taken of the two groups to observe differences.

The main investigation aimed to observe differences between control and injection sites at varying wool lengths by IRT. It was determined that three wool lengths: short (1-2mm) medium (5-10mm) and long (15-20mm) should be used to gain an insight into the ability of IRT to detect skin inflammation. This phase, conducted at AgResearch, New Zealand, involved the use of live sheep with patches shorn to the three fleece lengths to determine whether localised skin inflammation resulting from an intradermal vaccine formulation injection could be detected with IRT.

5.2.2 Validation observations – *Determining if differences in existing flystrike could be identified through IRT*

The initial observational phase of this investigation took place at the Kirby Farm facility at the University of New England in Armidale, NSW, Australia. Merino lambs aged 3-4 months were brought in to the animal yards for routine husbandry management and two struck animals and two controls were identified. The wool length was approximately 3cm on all the animals. Infrared thermography images were taken of the struck area and surrounding unaffected area as well as similar areas of the control animals and analysed to observe differences.

5.2.3 Animals

As the initial validation work only consisted of observations made during normal farm procedures no ethics approval was required or sought for this work. The main experiment was conducted under and with approval from AgResearch Animal Ethics Committee, AE number 13780.

Thirty-five (thirty for treatment and five spare sheep) New Zealand Romney sheep (2 tooth, 34 pregnant ewes and one wether) were brought into the animal holding facility two weeks prior to commencement of the experiment, and were habituated to handling and feeding. All sheep were healthy and in good body condition with mean body score 3.5 ± 0.5 s.e., and mean weight 58.75 ± 8.49 kg s.e. The sheep were housed in groups of 10 in pens in the animal facility. Pens were mesh floored and were cleaned daily. The animals were fed a lucerne pellet with a crude protein of 18-25% and fibre content of 20-25% (T Hale 2017, personal communication, 4 July) at a rate of 0.5 kg/animal along with grass hay *ad libitum*. Water was available at all times. Temperature and relative humidity loggers were placed around the pens and the holding facility to record ambient temperatures for the duration of the experimental period.

5.2.4 Main experiment: injection reaction testing

5.2.4.1 Part 1 – Determining likely appropriate wool length for IRT detection of skin inflammation.

A skin with fleece from a freshly slaughtered sheep was obtained from Wallace & Co abattoir and processing plant for investigation into wool lengths for testing. To observe the amount of heat transfer through the skin, the skin was stretched over a heat lamp and the wool was shorn to varying lengths. The heat lamp was positioned at a distance underneath the skin to mimic body temperature, resulting in a temperature at the skin surface of approximately 36°C. Several fleece lengths were tested and observations made regarding the most appropriate lengths to use which may provide a differential for the reflected heat from underneath the skin to be apparent. The shortest length showed a temperature that was as close as possible to skin temperature, the longest length showed a temperature reading closer to the ambient temperature, and the medium length showed a temperature reading approximately in the middle of the temperature measurements recorded at the short and long wool lengths.

Lengths of 1-2mm, 10-15mm and 15-20mm were chosen to represent “short” (S), medium (M) and long (L) wool lengths.

5.2.4.2 Part 2 – Measuring skin inflammation using IRT

An experiment was performed to determine if localised skin inflammation could be detected by IRT at various wool lengths.

5.2.4.2.1 Treatments

Animals were introduced into the animal facility seven (7) days prior to experimental work commencement for the purpose of familiarisation and acclimation to feed and handling. Any lame sheep were removed from the experiment and placed in spare pens to rest. Sick sheep were removed from the experiment and treated by the attending veterinarian. Injection and control patches were shaved on each animal on day -1. These lengths were shorn onto the 30 experimental animals and three spare animals in four patches of two lengths each, giving a total of 20 injection and 20 control patches for each of the three lengths: short, medium and long. Additionally, the 3 spare animals were also tested at the same time to allow substitutions to take place at any point during the experimental period. Each injection and control area was measured 10cm² and injection sites were diagonally opposite on each animal to reduce the impact of any inflammation spread see Fig. 5.1.



Fig. 5.1 Infrared image of sheep with injection patches (top left and bottom right) and control patches (top right and bottom left) with medium (top) and short (bottom) wool lengths.

The vaccine used to stimulate an inflammatory response was Covexin® 10 (Zoetis), a commonly used broad spectrum vaccine which provides protection for sheep from a range of clostridial diseases. This vaccine is associated with swelling at the injection site occurring in the majority of animals, with a mean value of 6cm (Irish Medicines Board 2009). An initial booster with the full dose of the vaccine (1mL) was administered subcutaneously on day -3 to all sheep to prime the immune system. To stimulate inflammation, a 0.5mm diabetes needle

was used to intradermally inject 0.1mL of vaccine formulation into the centre of each of the two injection areas on day 0 and day 7.

5.2.4.2.2 Measurements

Sheep were moved into a holding pen one group at a time at the commencement of each measurement period, and were then loaded individually into a crush and head bale. A food reward was placed into the head bale to reduce stress caused by handling and restraint. The animals showed a significant improvement in handling by day 2, becoming more willing to enter the head bale and showing reduced resistance to handling and restraint. The IRT camera was positioned one metre directly above the target area of the sheep's back, and an IRT image recorded. Air temperature and relative humidity measurement was also taken at the time of each measurement and image capture in order to assist in the correct calculation of air temperature as measured by the IRT camera. Following each measurement the animal was released to return to the pens and its companions.

5.2.4.2.3 Analysis

Thermal imaging was performed using infrared cameras, model B250 (FLIR Systems AB, Daneryd, Sweden) for the validation observations, and T650sc (FLIR Systems AB, Daneryd, Sweden) for the main experiment.

Statistical software R (v 3.3.1, 2016) was used for statistical analyses.

Analysis was performed on each image using R statistical software (R Core Team 2016). The raw 16-bit thermal images were extracted from the camera output. A 10cm² region of interest (ROI) was established in each image and the pixel values within averaged. A maximum pixel value was also obtained with the proviso that 0.1% of the pixels within the ROI reached that value before it was accepted. The raw values were then converted to true temperature using the R package "thermimage" ensuring manual measurements of ambient temperature and humidity were integrated along with the constants and reference values logged by the camera. The control and injection areas were then compared for both average temperature and maximum temperature.

A one-sided paired t-test comparison of the difference between means was performed to compare injection sites and control sites across individual animals and measurement days for each of the three wool lengths.

5.3 Results

5.3.1 Validation investigation

The struck areas showed an observed temperature difference range of +2 to -4°C compared to the same area on the control animals, with the struck areas showing a lower temperature than the control areas on the same animal. This investigation was limited due to the small number of struck animals available at the time of observations and therefore it is difficult to draw meaningful conclusions from the available information.

5.3.2 Main experiment

5.3.2.1 General observations:

Treatment areas showed palpable swelling at injection sites from day 1 of the experiment, by day 2 some heat spots can be seen on the IRT images on the short patches.

Significant differences ($P < 0.05$) were found across measurement days 1-3, 6, 8 and 10 for the short wool length (Table 5.). Control and injection sites at medium wool length showed significant differences on days 8, 9 and 10 ($P < 0.005$) and at long wool length significant differences were found on days 2, 3 and 5, however variation was greater at the medium and long lengths compared to the short length as seen in the standard deviation from the means in Table 5..

Table 5.1 Maximum temperatures of control and injection sites detected by infrared thermography at short, medium and long wool lengths over an experimental period of ten days.

Wool length	Day	Control mean $\pm$ s.d.	Injection mean $\pm$ s.d.	P - value
short	All	30.42 ^a $\pm$ 2.16	30.73 ^b $\pm$ 2.08	***
	1	32.75 ^a $\pm$ 1.51	33.18 ^b $\pm$ 1.23	**
	2	31.19 ^a $\pm$ 1.42	31.74 ^b $\pm$ 1.26	***
	3	30.01 ^a $\pm$ 1.90	30.57 ^b $\pm$ 1.50	**
	4	29.46 $\pm$ 2.15	29.76 $\pm$ 1.93	n.s.
	5	28.89 $\pm$ 2.20	28.92 $\pm$ 1.93	n.s.
	6	29.10 ^a $\pm$ 1.97	28.96 ^b $\pm$ 1.74	***
	8	29.57 ^a $\pm$ 1.63	30.11 ^b $\pm$ 1.63	***
	9	30.65 $\pm$ 0.96	31.20 $\pm$ 0.92	n.s.
	10	31.21 ^a $\pm$ 1.76	31.38 ^b $\pm$ 1.94	**
medium	All	25.48 ^a $\pm$ 2.68	25.64 ^b $\pm$ 2.77	**
	1	26.87 $\pm$ 2.56	27.03 $\pm$ 2.25	n.s.
	2	25.19 $\pm$ 2.17	25.25 $\pm$ 2.02	n.s.
	3	24.50 $\pm$ 2.38	24.51 $\pm$ 2.48	n.s.
	4	23.97 $\pm$ 2.82	24.06 $\pm$ 2.80	n.s.
	5	23.91 $\pm$ 2.75	23.71 $\pm$ 2.94	n.s.
	6	24.34 $\pm$ 2.54	24.24 $\pm$ 2.73	n.s.
	8	25.52 ^a $\pm$ 1.79	26.15 ^b $\pm$ 1.91	***
	9	26.98 ^a $\pm$ 1.13	27.61 ^b $\pm$ 1.28	***
	10	27.24 ^a $\pm$ 2.14	27.72 ^b $\pm$ 2.10	**
long	All	21.61 ^a $\pm$ 2.96	21.79 ^b $\pm$ 2.90	**
	1	22.85 $\pm$ 1.99	23.20 $\pm$ 2.22	n.s.
	2	21.47 ^a $\pm$ 2.13	21.62 ^b $\pm$ 2.12	*
	3	19.81 ^a $\pm$ 2.44	20.31 ^b $\pm$ 2.74	*
	4	19.48 $\pm$ 2.62	19.72 $\pm$ 3.08	n.s.
	5	18.68 ^a $\pm$ 2.75	19.24 ^b $\pm$ 2.90	*
	6	20.74 $\pm$ 3.80	20.23 $\pm$ 2.45	n.s.
	8	22.63 $\pm$ 1.24	22.78 $\pm$ 1.27	n.s.
	9	24.04 $\pm$ 1.06	24.23 $\pm$ 1.37	n.s.
	10	24.51 $\pm$ 1.16	24.39 $\pm$ 1.50	n.s.

Values are means ($\pm$ s.d.) and those with different letters in a row are different. *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

5.4 Discussion

Our results found that significant temperature differentials were detected between injection and control areas for the short wool length on 6 of the 9 trial days, suggesting that ready detection of inflammation at short wool lengths may be possible. However, the strength of the reaction may vary and be impacting on the thermal response at the skin, given that stronger, but more short-lived, responses were seen after the second intradermal injection was given. Significant differences were found across several measurement days for the medium wool length. However, temperatures at the individual sites varied more at medium and long wool lengths than at short, and this may have impacted on the ability of the IRT to obtain an accurate comparison of the temperature at the level of the skin. This increased variability is likely to be attributable to the varied characteristics of wool which may contribute to the reduced thermal transfer due to factors such as hair diameter and the crimping of the hair described by Cena (1973). Combining the results from all measurement days showed significant differences at each of the wool lengths, however again the increased variability may be impacting on these results at the medium and long wool lengths, and these results should therefore be treated with caution.

Limited detection of significant differences between control and injection sites on the long wool length patches suggest that it will be difficult to detect a temperature difference caused by inflammation. This might be expected given the insulation qualities of wool in preventing the thermal transfer of heat to and from the skin of the sheep (MacFarlane 1964). The insulating nature of fleece inhibits the transfer of heat from the skin to the surface of the fleece, and factors such as fleece density, quality and cleanliness will also impact on the variability of the surface temperature and the amount of heat which may be detected from the skin through IRT. The thickness of the skin of woolled areas may also play a role in the dissipation of heat from the injection site, with woolled areas of skin being thicker than non-wooled areas, and skin increasing in thickness after shearing, suggesting a role in thermoregulation (Williams and Thornberry 1992). Therefore, it is possible that the location of the inflamed site on the body may result in differences in temperatures as measured by IRT.

While a great deal of research has focused on the potential of IRT in detection of illnesses associated with inflammation, the literature on the mechanisms of the amount of radiant heat produced in association with inflammation is scarce, and the implementation and usefulness of IRT relies heavily on this association. It is unclear how much heat is produced when an area

becomes inflamed, and how much of this inflammation is a result of increased blood flow, or the accumulation of fluid in the inflamed area, or both. Infrared thermography is known to be utilised in the detection of blood flow (Stromberg and Norberg 1971) and is therefore a good indicator of increased blood flow to an area, however inflammation is not only characterised by increased blood flow, but also other cellular processes (Poljak-Blazi *et al.* 2009), with prolonged inflammation resulting in scar tissue formation (Kumta *et al.* 1994) which may in turn show reduced blood flow in the area. The cause of the inflammation may further complicate this process, as bacterial infections will also result in oedema (swelling) from plasma leaking from the capillaries, and dilation of the blood vessels (Hovinen *et al.* 2008). Martins *et al.* (2013) also makes reference to oedema not necessarily being associated with higher surface temperatures. In light of this, further investigation into the surface temperature of inflammation and the underlying mechanisms of inflammation associated with various illnesses and infections may be warranted as they pertain to the use of IRT in the detection of skin inflammation.

It is possible that the vaccination reaction generated was not strong enough to produce a distinct heat signal which could be detected through the longer wool lengths, further investigation in this area may be warranted using a stronger, more well-known inflammatory agent, such as carrageenan (Calkosinski *et al.* 2015) or turpentine (Colditz *et al.* 2011).

The remote detection of clinically significant inflammation at short wool lengths could provide useful information regarding injuries and skin pathologies in recently shorn animals, reducing unnecessary handling and allowing for earlier detection and treatment of illnesses and injuries in extensively raised sheep. Additionally, the ability to detect moisture on the surface of long wool, as shown in the preliminary investigation, may have utility in the indirect detection of fly strike and the associated risk factors (Watts *et al.* 1979).

5.5 Conclusion

These findings show that detection of inflammation may be possible at short wool lengths, this may have application in the remote detection of skin inflammation for a short time after shearing and at short wool lengths. The IRT technique appears to have limited application at longer wool lengths in the detection of inflammation of the skin; however, it could have utility in detecting risk factors for flystrike such as higher levels of moisture on the surface of the fleece or the skin. Further investigation may be warranted into the potential for IRT use in remote monitoring systems for sheep.

Chapter 6

General discussion

6.1 Introduction

The objective of this thesis was to investigate the potential of remote technologies in monitoring sheep health. The literature review undertaken as part of this thesis (Chapter 2) outlined the current knowledge and need for further investigation into emerging technologies, with remote temperature monitoring showing the most promise for objective animal health measurement utilising currently available devices. Two temperature measurement devices (temperature-sensing microchips and infra-red thermography) were examined in terms of their applicability in body temperature monitoring, and the detection of inflammation in sheep. The potential of IRT as a peripheral predictor of core temperature has been investigated by Chung *et al.* (2010) and Cook *et al.* (2015) in pigs, and has been studied in the detection of inflammation in non-wooled areas in sheep (Martins *et al.* 2013), however its potential in the detection of inflammation under wool has not been explored. The investigation of these measures in this thesis addresses some of the questions which arise in the development of remote monitoring tools, such as the impact of endogenous rhythmicity on peripheral temperature measures, also the impact of feeding frequency on rhythmicity, and finally the impact of fever both on rhythmicity and the reflection of same in peripheral measures. Temperature sensing microchips were investigated in two peripheral locations, the neck muscle (sternocleidomastoid muscle) and subcutaneously under the tail; these were investigated in terms of their ability to reflect core temperature patterns under normal conditions (detailed in Chapter 3) and under fever challenge (detailed in Chapter 4). It was found that the neck microchip most closely followed the core temperature patterns under normal conditions, and the tail microchip was least reflective of core temperature patterns. Chapter 5 then goes on to explore the potential application of infrared thermography (IRT) in the detection of skin inflammation at varying lengths of wool. The ability of IRT to detect skin inflammation at three wool lengths was tested, out of the three lengths (short, medium and long) inflammation was only reliably detected at the short wool length. It was determined that both devices have possible utility in the development of a remote monitoring system, as well as providing new insights into the thermoregulatory influences on body temperature measurements using these devices. The investigation of these measures in this thesis therefore addresses some of the questions which arise in the development of remote monitoring tools.

6.2 Application of neck microchips in a remote health monitoring capacity

With the information gained in our results, it is possible to then outline the steps which may be necessary to advance this technology into a practical application in remote monitoring. This would likely involve the development of an algorithm in which body temperature measurements could be incorporated to develop a “baseline” and, by extension, then be utilised to “flag” a possibly abnormal state based on the analysis of the measurements taken. In order to develop such an algorithm for the detection of a disease state in animals based on peripheral measurements, it is important to consider the factors which impact on peripheral temperatures. These factors include (but are not limited to): ambient temperature, circadian rhythms, activity levels including feeding activity, feeding frequency and feed consumption, sleep-wake patterns, the presence or absence of external time cues such as daylight, metabolic heat production and age of the animal, many of which have their own circadian rhythms which may or may not be synchronous with body temperature, but which can influence normal body temperature measurements and patterns (Refinetti and Menaker 1992). Heat production within the muscle also requires consideration due to the potential impact of heat produced through activity on the muscle measurement obtained through the microchip (Ardévol *et al.* 1998). Further research may be needed to investigate the influence of some of these factors on the circadian rhythm of body temperature as they relate to extensively managed sheep.

The analysis in Chapter 3 outlines the differences seen in core temperature due to feeding schedule and peaks associated with feeding activity. While these absolute temperature variations are within the normal temperature range of sheep ($\sim 1^{\circ}\text{C}$), the timing of peaks associated with feeding activity can be used to inform an investigation on the basis of the temperature differential which may be expected to be seen under normal (healthy) conditions, which could then incorporate a feeding peak which may be expected due to the timing of a feeding bout, this could then be discounted as an alert providing it is within the normal temperature range which might be expected to coincide with feeding activity (i.e. $<1^{\circ}\text{C}$). The observed temperature range may vary in animals grazing in an extensive production system and would therefore require further research for use in this context. The trend observed in experiment 2 in Chapter 3 suggest that smaller amounts spread over the day results in smaller peaks of shorter duration associated with the feeding activity. It was also observed that peaks associated with feeding activity were no longer than 0.5hrs in duration, therefore any peak temperature being sustained for longer than that may be able to be flagged as abnormal in a confined situation, the application of this knowledge in an extensive grazing environment requires further investigation, both in the context of disease detection and also under a variety

of environmental conditions such as adverse weather and seasonal differences. Thresholds of disease states may then be able to be set based on these factors. For example, it is known that basal temperature in the late afternoon is likely to be higher by as much as 1°C than temperatures in early morning, due to the normal rhythmicity of body temperature (Bligh *et al.* 1965), and our results concur with this knowledge, showing mean 24-hour range of oscillation at 0.96°C for vaginal (core) temperatures and a strong diurnal pattern lower in the early morning and higher in the late afternoon. Therefore any “threshold” temperature for disease states must take into account this variation and correct for it when designing an algorithm which “flags” a suspect temperature that is outside of the normal expected range for that animal. Additionally, Bligh *et al.* (1965) found an additional 0.8°C variation which is thought to be attributable to seasonal variations, the shorter time frame of our experiment did not allow us to observe a seasonal variation, and therefore this is an additional factor which needs to be considered in future research.

Disease states have been associated with alteration in several circadian rhythm parameters (Refinetti and Menaker 1992), therefore any algorithm design must incorporate the calculation of the key parameters characterising circadian rhythms: period, shape, amplitude, mean level and phase. Additionally, the absence of a discernible circadian rhythm may also be indicative of some pathology, for example the circadian rhythm disappeared in animals suffering from foot rot (D'Alterio *et al.* 2012): this characteristic can be used as a diagnostic tool in the determination of illness or disease in sheep. The frequency of measures will influence the utility of the data collected in a remote setting. The presence or absence of a rhythm was described using measures obtained at dawn and dusk over a period of 30 days in horses and sheep (Piccione *et al.* 2002b). Detection of changes to the parameters or pattern of change would require more frequent measurements, particularly if circadian data transformation models are used. The exact frequency of measures to accurately detect clinically significant change is not yet known and further research is recommended to assess the effectiveness and applicability of these methods of analyses in the remote detection of body temperature change in sheep.

The analyses undertaken in Chapter 4 demonstrate the significance of pattern change over the duration of the effect of the lipopolysaccharide injection, with a change in temperature pattern lasting 3-4 hours as detected by the core temperature measure and the neck muscle microchip measure, the tail measure detected a change of less than 2 hours. Thus, a significant change in

temperature pattern for a period of time longer than 2 hours may be indicative of a pathological impact on body temperature or the activation of a fever response and this could then be incorporated into these calculations. When incorporating these factors into an algorithm it is also necessary to include other information, such as weather conditions, feed availability, and predation events, all of which will have an impact on both body temperature and rhythmicity. Some of the factors which need to be considered are outlined in the following section.

With regard to the application of microchip technology in extensive production systems further work is likely to be required, particularly in consideration of their use in establishing body temperature patterns over a 24-hour period. Currently the microchips have no way to store information and only offer a temperature at the time the information is read by a reader, if incorporated into a walk-over-weigh system, for example, it is likely to only obtain as many measurements per day as the animal is required to walk past the reader, at a controlled movement point such as near water. Additionally the strength of the reader antenna may need to be increased in order to improve the reading range to reduce the requirement for the animal to be very close to the reader. While there are limitations in the application of this technology, work is underway to improve the reliability of monitoring technologies for use in extensive systems such as walk-over-weighing which may allow the implementation of a temperature measurement component. Frequency of measures would need to be determined in order to achieve a reliable indication of normal temperatures, depending on the timing a circadian rhythm can be observed with measures obtained at dawn and dusk, and this information may be utilised to build a profile of “normal” body temperature patterns for the individual animal.

6.3 Physiological and environmental influences on core and peripheral body temperature measures.

The influence of circadian rhythmicity in peripheral temperature measurements requires extensive investigation in the context of disease states and disease detection. Previous work using technologies such as microchips and infrared thermography is limited in the context of illness and disease, particularly in sheep which present unique challenges due to the insulating properties of wool.

Much of the existing research has focused on the endogenous or exogenous regulation of circadian rhythmicity in peripheral temperatures (Brown *et al.* 2002; MacLeay *et al.* 2003; Menaker *et al.* 2013). Previous research has examined the factors affecting peripheral temperature measurements and have used anaesthetised animals (Tomasic and Nann 1999;

Mansel *et al.* 2008), the temperature differentials observed between central and peripheral locations (Tang and Kiyatkin 2010) and the role of different physiological regions in generation of the fever response (Harden *et al.* 2014). Less research in animals has looked at the utilisation of peripheral measures in health monitoring. Tomasic and Nann (1999) observed the use of peripheral temperature measurement in anaesthetised horses, and D'Alterio *et al.* (2011) observed the pattern of foot temperature in sheep to observe the difference between exogenous and endogenous control of circadian rhythmicity. Lohse *et al.* (2010) investigated the utility of temperature sensing microchips in illness but determined their effectiveness was limited in a clinical capacity due to their inability to reflect exact core temperature. While our results showed a similar disparity from absolute core temperature, we examined the ability of peripheral sensors to reflect core temperature rhythmicity, as deviation from this pattern may also be utilised in a clinical or diagnostic capacity. The role of circadian rhythmicity in detection of disease states relates strongly to peripheral measures. Since peripheral measures do not reflect absolute core temperatures, it is more important to ensure that rhythmicity and fever states are accurately reflected to determine whether these peripheral measures have utility in the detection of a change in an animal's internal state. As demonstrated in Chapter 3, the importance of detection of pattern of change can be seen when comparing measurement locations, and in Chapter 4 the clinical and diagnostic significance of the detection of pattern change is significantly impacted by the location of the measurement device, with the neck microchip showing a marked similarity to the core temperature change observed, while the tail failed to detect significant temperature change caused by the introduction of the lipopolysaccharide. Consequently, the ability of the device to inform about the thermophysiological state of the animal is dependent on its ability to detect both changes of clinical significance and changes in the daily rhythmicity.

While previous work has focused on the factors related to endogenous rhythmicity such as the hypothalamic control centres investigated by Menaker *et al.* (2013) and Saper (2013), and metabolic control outlined in Haydon *et al.* (2013), our work begins to look at the impacts of external factors, such as feeding behaviour and rate of feeding. Previous work has focused largely on ration amount in the context of its impact on body temperature and rhythmicity, looking at feed efficiency (DiGiacomo *et al.* 2014; Nikkhah 2014), feed type (Jacob *et al.* 2014) and feed amount (Maloney *et al.* 2013). Our work examining the impact of different feeding regimes, that is feeding at the same ration amount but split into 2, 3 and 5 feeds spread throughout the day, is detailed in Chapter 3, and observes the effect on the circadian rhythm.

This research compared the circadian rhythm of body temperature observed under normal conditions, with that of the same circadian rhythm using data which had temperature spikes associated with feeding activity removed, in order to investigate the impact of that feeding activity on the rhythmicity of body temperature.

It is known that different areas of the body play a role in thermoregulation (Baldwin 1975; Bligh 1979; Brigg *et al.* 1994). This thesis investigates how this impacts on the peripheral locations which have been chosen, and how it impacts on the ability of peripheral measures to reflect core temperatures and core temperature rhythmicity. Results shown in Chapter 3 demonstrate the marked differences in the mean variation (expressed as the circadian parameter “range of oscillation”) observed over a 24-hour period between the vagina (0.96°C), neck (1.57°C), tail (2.59°C). With the skin playing a significant role in thermoregulation (Cockrem and Wickham 1960) this explains both the high degree of variation seen in the tail measurements, and also the influence of ambient temperature on this area. Previous work using temperature sensing microchips in sheep showed significant correlations between temperatures from microchips placed in the nuchal ligament, and rectal temperatures, with variation up to 0.5°C (de Oliveira *et al.* 2010) with no thermal challenge tested. Work with rats and mice was examined under *Klebsiella pneumoniae* infection (Kort *et al.* 1998), measurements in mares to detect changes associated with parturition (Korosue *et al.* 2012), and cats experimentally infected with FHV1 (Quimby *et al.* 2009), showing that the microchip transponders may be effective in this capacity.

In Chapter 4 we tested the ability of peripheral temperature measures to reflect core temperatures under both normal conditions and under challenge and found that microchip transponders can show a significant temperature differential from core temperatures, with microchips placed in the neck muscle around 2°C lower than core temperature, and microchips placed subcutaneously under the tail reading around 3.5°C lower than core temperature, this is consistent with other work on peripheral temperatures with transponders (Goodwin 1998; Lohse *et al.* 2010). This variation was consistent following the response to lipopolysaccharide administration, suggesting that these temperature differentials may also be seen under fever conditions. However, the pattern of change across the day was more closely reflected in the neck microchips when compared to core temperature than the tail. Our work suggests that the placement of the temperature measurement devices influences the effectiveness of the measurement in detection of temperature change, particularly as it compares to core temperature measures.

Although some work has demonstrated that some measures reflect core temperature depending on circumstances, species and measurement locations (Goodwin 1998; Quimby *et al.* 2009; de Oliveira *et al.* 2010), much of the existing research is consistent in demonstrating that peripheral measures are significantly different from core absolute temperatures (Robinson *et al.* 2008; Lohse *et al.* 2010; Brunell 2012). For example in work conducted by Reid (2014) it was demonstrated that a number of peripheral measures undertaken using temperature sensing microchips were unsuitable in detection of temperature change associated with lipopolysaccharide injection. However, these findings do not imply that peripheral temperatures are not useful in monitoring the health of sheep in a remote setting, but further research is needed to refine these techniques and confirm the optimal placement of sensors. The detection of temperature change within an individual along with any significant deviation from the normal variation that can be seen in a healthy animal will inform researchers as to the efficacy of these measures.

Therefore, if peripheral temperatures can reflect the rhythmicity that is seen in core temperatures, might they also be useful in detection of clinically significant changes in rhythmicity? Our research demonstrates that the answer may depend on the location of the peripheral measurement. Using temperature sensing microchips, the neck muscle location appeared to reflect core temperature rhythmicity more closely than the microchip placed subcutaneous tail measure. There are several possible reasons for this; subcutaneous measures are far more likely to be subject to factors such as ambient temperature and thermo-regulation, as the skin interface plays a major role in thermoregulatory processes. A great deal of heat transfer occurs at this the level of the skin, and the skin under the tail is thinner than that of woolled areas, as well as being subject to surface influences such as movement and the presence of moisture. Additionally, the properties and features of the skin varies according to its location, whether it grows hair or wool (Lyne and Hollis 1968), and even the breed of the animal (Williams and Thornberry 1992). Thus, these measures are highly variable, and this variability impacts on the ability of this measure to accurately affect the rhythmicity of core body temperature, and subsequently whether this impacts on its ability to detect clinically significant changes in both absolute temperature and changes in the circadian rhythm. This phenomenon was demonstrated in experiments looking at both normal temperature rhythm (Chapter 3), and abnormal (fever) temperature patterns (Chapter 4). The ability to distinguish between normal and abnormal body temperature is critical for the application of this technology in remote temperature measurement. Limitations in the application of this technology in the field may

include factors such as placement, muscle is a useable part of the animal that has value in sheep meat production. Therefore, the neck is not as desirable from an industry perspective as the tail which has little to no production value. Consequently, if the tail is more useful to industry, then more work needs to be done to investigate ways to reduce the impact of the variation interfering with the ability of this measurement to accurately reflect core temperature patterns both under normal conditions and under thermal challenge. Other avenues of investigation could include more effective recovery of the microchips so that the neck muscle can still be utilised in production, previous research suggests this may be problematic (Hunt 1994; Huber 2004; Caja *et al.* 2005). Additionally, further work in the development of remote reading needs to be undertaken, currently available readers are unable to take consistent readings in a remote capacity, improving the read range and antenna capabilities of these readers is necessary in order to maximise the potential of this technology.

6.4 Infra-red thermography in the detection of skin inflammation in sheep.

Infrared thermography (IRT) has potential in the remote monitoring space. Research is ongoing in the use of IRT in body temperature measurement, detection of inflammation in feet in sheep (Talukder *et al.* 2015) and cattle (Alsaad *et al.* 2015); and udders in sheep (Martins *et al.* 2013) and cattle (Colak *et al.* 2008; Hovinen *et al.* 2008). However, in sheep the presence of wool can be problematic, due to the highly insulative properties of wool, which can result in a significant temperature differential at the surface of the wool compared to the skin surface (MacFarlane 1964). Therefore, it is important to determine whether skin temperatures that vary from normal can be differentiated despite the presence and thickness of the wool. The variability of temperature observed at the wool surface demonstrates the difficulty in differentiation of the inflamed area of the skin from the control area of the skin. Our results show that it is unlikely that areas of skin inflammation can be differentiated when wool is longer than 2mm (Chapter 5). However, the results obtained in our study may have been limited by the inflammatory agent used, vaccinations such as the one used often cause swelling at the injection site (Irish Medicines Board 2009), however it is not known how strong the heat reaction produced would be, and it is possible that a stronger inflammatory agent may have resulted in a larger temperature differential between the injection and control sites at longer wool lengths, further investigation into this may prove useful. These results do not exclude IRT from application in the remote detection of conditions such as fly strike, as they can result in a temperature differential due to factors other than inflammation. Initial validation work showed a temperature differential for struck areas of sheep which maybe attributable to the presence of

moisture at the surface of the wool, possibly due to evaporative cooling of the exudate from the irritation of the skin, however there is insufficient data to determine whether this is common across a variety of cases of flystrike. The presence of a temperature differential such as that caused by moisture at the surface may have some utility and warrants further investigation. The persistent detection of moisture on the wool surface could indicate fly strike due to the exudate from the affected skin or other injury, and could have application in a remote monitoring system. Additionally, the detection of inflammation at short wool lengths may also have application in the detection of injuries and other health issues for a period of time in lambs and after shearing. However, control of the environment is an important factor in the application of infrared thermography in the field, and further research is needed to incorporate the automation of image capture, along with improved focus in the case of animals moving at the times that the images are taken.

Chapter 7

Conclusions

7.1 Conclusions

In conclusion, the application of remote temperature sensing in extensive sheep production has potential utility in identification of unwell animals. This thesis has demonstrated a greater understanding of the role of peripheral locations in body temperature measurement, and examined some of the factors which may impact on these measurements. However, implementation of this technology requires careful consideration of factors which influence the efficacy of those measures. Further investigation into the efficacy of microchips in detection of clinically significant temperature change needs to be undertaken, particularly under field conditions and differing environmental conditions, as well as observing other states which may influence the circadian rhythmicity of body temperature, particularly as they may apply to peripheral measurement locations. These implications should be considered when looking to develop an algorithm for the detection of clinically significant temperature change in an individual. It is also recommended that implications of physical activity and metabolic heat production be investigated as they may also influence the temperature at the site of the neck muscle. Additionally, work investigating the utility of microchips in a production capacity and the influence of these on carcass values should also be considered.

The investigation of the potential of infrared thermography must take into account the insulating nature of wool and its limited ability to detect inflammation at wool lengths greater than ~2mm. Investigation into the use of infrared to detect fly struck animals through the presence of moisture on the surface of the wool is recommended to be undertaken in order to fully understand the potential utilisation of infrared thermography in the remote detection of fly strike.

By taking these factors into consideration it is possible to develop a remote temperature monitoring methodology and application in extensive sheep production systems as part of an integrated remote health monitoring system. This thesis provides insight into the application of various remote monitoring technologies and how they may be applicable in animal health monitoring in extensive production systems, as well as investigating some of the thermophysiological impacts on temperature measurement and analysis.

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