Factors associated with the housing environment of calves in relation to the prevention of respiratory disease

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Declaration

I declare that I have composed the present thesis and that the work described is my own with assistance received acknowledged. The work has not been submitted for any other degree or professional qualification.

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Abstract

Respiratory disease is a multifactorial disease that is a major cause of morbidity and mortality in calves. The main emphasis of this thesis is surrounding the housing environment and its effect on the calf during the pre-weaning phase of life. The housing environment can affect disease levels as well as general performance in calves. An important physical aspect of calf housing is ventilation as it allows the removal of airborne microorganisms, particulate matter and moisture from the air. In the first instance, the best method is to prevent the generation of such fomites. In this work, the factors of the quality of straw bedding material and presence of calves were analysed for the outcomes of particle and total bacterial count. Results showed that the presence of calves can increase the levels of particles and total bacterial count. This study showed that there was no effect of straw quality on particulate matter and total bacterial count within the range of straw types used.

Within the calf housing, there can be areas of the microclimate that can cause the calf to display a response such as removing itself from the hostile environment. This behavioural response was examined in relation to specific air temperature (5^{o}C, 10^{o}C, 15^{o}C) and wind speed combinations (0m/s, 1m/s, 3.3m/s). Overall it was found that calves showed an aversion to increasing wind speed and that there was no significant effect of air temperature on any of the behavioural measures.

The calf is exposed to naturally occurring variations in air temperature, wind speed and air quality on a daily, and occasionally hourly, basis during this vulnerable period of life. The ramifications of being exposed to such variations in terms of calf growth in the first month of life were examined in a longitudinal study of 299 dairy and dairy cross beef calves over one year. A longer period of exposure to temperatures below the lower critical temperature for calves had a significant effect on the daily liveweight gain.

The variation in environmental conditions provides potential stressors to the young calf which can increase the susceptibility to disease. An indicator of respiratory disease is an elevation in core body temperature which is normally assessed by taking a rectal temperature measure. As part of this work, a non-invasive method for temperature acquisition was explored. The association between rectal temperature and the temperature obtained from thermal imaging the area surrounding the medial canthus was established as being weak. Despite incorporating the environmental factors that influenced the thermal image temperature (air temperature and wind speed) into a predictive model, there was only a very minor improvement in the relationship but not enough to establish thermal imaging as a reliable method for obtaining the core body temperature of calves. Although repeatable, the temperature gained from thermal imaging may phenotypically not reflect core body temperature.

In conclusion, this thesis has provided supporting evidence to accentuate specific aspects of calf housing such as protection from draughts at calf level and the identification of risk factors in terms of air quality. It also highlights the impact the housing environment can have on the growth of the calf pre-weaning.

Lay Summary

One of the main causes of illness and death amongst calves is respiratory disease. Respiratory disease is a multifactorial disease which means that factors such as the environment in which the calf is kept, the bacteria and viruses that cause the disease and the management of the calf all interact with one another leading to the disease. This thesis concentrates on one of these factors – the housing environment. The housing environment can affect disease levels as well as general performance in calves and an important physical aspect of calf housing is to have good ventilation. The role of ventilation is to remove stale air from the housing and replace it with a supply of fresh air. The stale air being removed contains particulate matter. Particulate matter is made up of flakes of skin, hair and dust from bedding material and feeding. In this work, the presence and quality of the straw bedding material and the presence of calves were analysed for their effect on four specific particle sizes as well as total bacterial count. Results showed that the presence of calves can increase the levels of particles and total bacterial count. This study showed that there was no effect of straw quality on particulate matter and total bacterial count within the range of straw types used.

There can be areas of the calf housing where the environment can differ from others, for example colder and draughtier. The behavioural response of the calf towards specific air temperature (5^{o}C, 10^{o}C, 15^{o}C) and wind speed combinations (0m/s, 1m/s, 3.3m/s) was studied. Overall it was found that calves showed an aversion to increasing wind speed and that there was no significant effect of air temperature on any of the behavioural measures.

Air temperature, wind speed and air quality naturally vary within each day and across days. These variations can impact the growth of the calf during the early stages of life. A year-long study was carried out looking at the

effect of the proportion of time calves were exposed to environmental temperatures that were below the lower critical temperature on the daily liveweight gain of the calves. The lower critical temperature is the temperature at which the calf needs to increase its heat production by either removing itself from that area or by increasing its energy intake. A high period of exposure to temperatures below the lower critical temperature for calves had a significant effect on the daily liveweight gain.

The variation in environmental conditions provides potential stressors to the young calf which can increase the susceptibility to disease. An indicator of respiratory disease is an increase in core body temperature. Normally, this would be assessed by taking a rectal temperature but as part of this work, a non-invasive method for temperature acquisition was assessed, namely thermal imaging. A weak association between rectal temperature and the temperature obtained from thermal imaging the area surrounding the inner eye was established. Thermal imaging uses the heat given off by an object and converts it into an image where different colours represent different temperatures. Incorporating air temperature and wind speed into a predictive model along with thermal image temperature only produced a very minor improvement in the relationship with rectal temperature. This improvement was not enough to establish thermal imaging as a reliable method for obtaining the core body temperature of calves.

Implications for calf management based on this thesis would be to pay close attention to the early days of a calf's life as the environment can affect its development.

In conclusion, this thesis has provided supporting evidence to accentuate specific aspects of calf housing such as protection from draughts at calf level and the identification of risk factors in terms of air quality. It also highlights the impact the housing environment can have on the growth of the calf pre-weaning.

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Publications

D.J. Bell, A.I. Macrae, M.A. Mitchell, C.S. Mason, A. Jennings, M.J. Haskell (2020) Comparison of thermal imaging and rectal temperature in the diagnosis of pyrexia in pre-weaned calves using on farm conditions. *Research in Veterinary Science. Volume 131. Pages 259-265.* https://doi.org/10.1016/j.rvsc.2020.05.004

Conference Proceedings

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Bell D. J., Macrae A. I., Jennings A., Mason C. S., Mitchell M. A., Haskell M. J. 2019. Behavioural response to temperature and wind in dairy calves. *Advances in Animal Biosciences- Animal Science Fit for the Future. Proceedings of the British Society of Animal Science, Dublin, Ireland.*

Bell D. J., Haskell M. J., Macrae A. I., Mitchell M. A., Jennings A., Mason C. S. 2019. Behavioural response of dairy calves to temperature and wind. *Proceeding of the 53rd Congress of the International Society for Applied Ethology, Bergen, Norway*.

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List of Abbreviations

AHDB	Agriculture & Horticulture Development Board
B2G	Birth until leaving the individual hutch
BRD	Bovine Respiratory Disease
BRSV	Bovine Respiratory Syncytial Virus
cfu/m^3	Colony -forming units per cubic metre
CMR	Calf milk replacer
cmr/d	Calf milk replacer per day
CV	Coefficient of variation
d	Days
DEFRA	Department for Environment, Food and Rural Affairs
df	Degrees of freedom
DLWG	Daily liveweight gain
DM	Dry matter
FTE	Full-time equivalent
g/d	Grams per day
g/kgDM	Grams per kilogram dry matter
g/m^3	Grams per cubic metre
G2E	Entering group housing Igloo pen until end of study
h	Hours
h/d	Hours per day

ID	Identification
IPCC	Intergovernmental Panel on Climate Change
Kg	Kilograms
kg/d	Kilograms per day
km/h	Kilometres per hour
l	Litres
l/d	Litres per day
Lat1Place	Latency to 1st movement between test pen and shelter pen
Lat1React	Latency to 1st behavioural reaction in test pen
LCT	Lower critical temperature
m/s	Metres per second
MADF	Modified acid digestible fibre
ME	Metabolisable energy
mins	Minutes
MPN	Most probable number of colonies
MPNC	Most probable number of colonies per cubic metre
NADIS	National Animal Disease Information Service
NE	Net Energy
NPV	Negative Predictive Value
PI-3	Parainfluenza – 3 virus
PPV	Positive Predictive Value
PropTP	Proportion of time in test pen

RH	Relative humidity
s	Seconds
sd	Standard deviation
se	Standard error (of mean)
Sn	Sensitivity
Sp	Specificity
SRUC	Scotland’s Rural College
TBehTP	Total number of behaviours expressed in test pen
TCZ	Thermal comfort zone
THI	Temperature - Humidity Index
TNZ	Thermo-neutral zone
UCT	Upper critical temperature
UK	United Kingdom
US	United States (of America)
VIC	Veterinary Investigation Centre
WVD	Water vapour density

Chapter 1: Literature Review

1.1 Introduction

In simple terms, the calves born today are the milking herd and beef production of tomorrow. However, 14.5% of live-born heifer calves fail to reach that stage of life which has major implications on the economics of the cattle industry (Brickell et al., 2009). One of the main reasons for this high calf mortality is disease in the pre-weaning stage.

In 2014, the Cattle Health and Welfare Group (CHAWG) identified calf mortality as a major cause for concern from a welfare and economic perspective. Studies have estimated dairy calf mortality rates in the United Kingdom (UK) to be in the region of 6% (Hyde et al., 2020) and 7.9% (Brickell et al., 2009). This UK figure is on par with Jorgensen et al. (2017) where it was reported that the calf mortality rate in the US is 6 to 8%. However, these figures do not compare favourably with calf mortality rates from Scandinavian countries. Svensson et al. (2006) reported in their study that 3.1% of calves died in Sweden between 1 and 91 days of age. Gulliksen et al. (2009) state that from their survey on Norwegian dairy herds, calf mortality rates were 4.6% amongst live-born calves which is reasonably similar to the calf mortality rate in Sweden. Gulliksen et al. (2009) believe the reason for this is that both countries (Norway and Sweden) have a high-health disease status. However as they correctly state, comparing calf mortality rates between different countries is very subjective due to the definition of calf mortality used. Overall, the UK has room for improvement in terms of calf mortality, not only in comparison with other countries but also between UK farms. Brickell et al. (2009) found the level of calf mortality ranged from 2% to 14% between farms.

Regardless of each country's definition of calf mortality rates, enteric and respiratory diseases are the major causes of calf deaths (Windeyer et al., 2014) and also calf morbidity. In a study carried out by Windeyer et al. (2014), 630 calves from a study population of 2784 (21.9%) were treated at least once for bovine respiratory disease highlighting that respiratory disease is a major cause of disease amongst calves.

The underlying theme of this thesis will be based around bovine respiratory disease, with the main focus surrounding the calf housing environment. The literature review aims to:

- provide an overview of bovine respiratory disease (BRD) and the consequences of the disease on the calf
- look at the approaches to reduce BRD through improved detection methods
- cover the basic principles of thermal comfort
- examine aspects of managing the housing environment

For the purpose of this thesis a calf will be defined as a bovine animal under six months of age in accordance with Council Directive 2008/119/EC, but more specifically a calf (artificially reared) in the pre-weaning stage of life (up to twelve weeks of age).

1.1 Bovine Respiratory Disease (BRD)

1.1.1 Description of disease

Bovine Respiratory Disease (BRD) has a large financial impact on the UK cattle industry. It is estimated to cost approximately £60 million per annum (Statham, 2011) and an average of £43.26 per case per dairy calf (Andrews, 2000) which was made up of veterinary and medicine costs, mortality, labour costs and weight loss. It is a major cause of morbidity and mortality in dairy calves. Studies by Donovan et al*.* (1998) and Sivula (1996) suggest respectively that 7.6% and 21% of dairy heifer calves will experience respiratory disease. A cross-sectional study by Walker et al. (2012) examined the morbidity and mortality rates for different calf rearing operations within the United States, and found that the morbidity rate for respiratory disease in calves at an operation level (i.e. farms which either reared female dairy calves only or those which raised both male and female calves) was 5.3% and the mortality rate was 0.47%. At the calf level, the morbidity and mortality rates were 17% and 1% respectively. The study also looked at the effectiveness of treatment for respiratory disease that was diagnosed and found that the case mortality rate on an operational level was 9% and at calf level it was 7.5%. This case mortality rate was in the same region as Sivula (1996) who reported this as 9.4%.

Many peoples' interpretation of the term BRD is that it is synonymous with pneumonia, but pneumonia specifically refers to the inflammation of the lungs. BRD, however, is the more appropriate terminology to describe the clinical syndrome where pathological changes are not necessarily confined to the lungs but the classical clinical signs (described later on) are present. Bovine respiratory disease is known as a multifactorial disease. This is because numerous environmental and management risk factors (stressors), along with infectious agents (Table 1.1), contribute to the development of disease. The main environmental risk factor relevant to respiratory disease is related to housing.

A review by Dennis (1986) indicated that housing modifies the climate surrounding the animal and can increase the concentration of pathogens in the air. The review also states that any stressor which lowers resistance will be augmented by housing conditions and environmental factors such as temperature and aerial pollutants like dust are important. Gorden and Plummer (2010) make reference to management risk factors being components such as stocking density, bedding type and the presence of older animals in the same air space as the young calf.

Ames (1997) reports the findings of various studies, for example Bryson (1985), into the causal agents and states that the main causes are Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza-3 virus (PI-3), *Mycoplasma bovis* and *Pasteurella multocida*. Initial signs of the onset of BRD can be non-specific and include elevated body temperature (over

39.5°C), an increase in respiratory rate, discharge from the eyes and nose, dullness and reduced feed intake (Lorenz et al., 2011).

Table 1.1 Infectious agents of bovine respiratory disease (BRD) present in the UK and other countries worldwide (Source: Andrews et al., 2004)

Mycoplasmas	**Viruses**	**Bacteria**
		Mannheimia (Pasteurella)
Mycoplasma bovirhinis	Respiratory syncytial virus (BRSV)*	haemolytica*
M. dispar *	Parainfluenza virus II	P. multocida*
M. mycoides subsp		Arcanobacterium(Actinomyces,
mycoides	Parainfluenza virus III (PI3)*	Corynebacterium) pyogenes*
(little importance in		
Europe)	Reovirus types 1,2,3 (Reo)	Streptococcus pneumoniae
M. alkalescens	Bovine viral diarrhoea virus (BVDV)*	Staphylococcus aureus
M. arginini	Adenovirus types 1,2,3,4	Strep. Bovis
M. bovis*	Enterovirus	Staph. Epidermidis
M. canis	Rhinovirus type 1	Strep. Faecalis
	Infectious bovine rhinotracheitis	
M. bovigenitalium	(IBR)*	Aerococcus viridans
Acholeplasma laidlawii	Respiratory bovine coronavirus	Acinetobacter spp.
A. modicum		Micrococcus luteus
A. axanthum		Staphylococcus spp.
Ureaplasma spp.*		Neisseria spp.
Ureaplasma diversum		Chlamydiales spp.
Leach's group 7		
mycoplasmas		Actinobacillus lignieresii
		Klebsiella spp.
		Corynebacterium bovis
		C. xerosis
		Streptococcus spp.
		Aerococcus spp.
		Haemophilus spp.
		Haemophilus somnus*
		Aeromonas spp.
		Bacillus spp.
		Alcaligenes faecalis
		Micrococcus roseus
		Micrococcus spp.
		Escherichia coli
		Fusobacterium necrophorum*

* indicates most important causes

By this stage, the disease will have already had an impact of the calf such as a reduction in daily liveweight gain (DLWG) and increased risk of death.

Infected calves disseminate these infectious agents through secretions from the nose, and also in the aerosols they produce when coughing (Bryson, 1985). Therefore the spread of disease can be via nose-to-nose contact and circulation of agents in the air. An appropriate intervention would therefore be the removal of the infected calf from the housing to reduce the spread of disease amongst other calves. However, as previously stated, by this stage the disease will have made an impact on the calf and potentially spread to others within that group/housing so the removal of the known infected calf may be too late.

1.1.2 Consequences of BRD

There is evidence to suggest that if the calf is fortunate to survive an episode of BRD, then there are some long-term consequences in later life. One such consequence of BRD at an early age is a reduction in growth rate (Van Der Fels-Klerx et al., 2002; Teixeira et al., 2017). The study by Teixeira et al. (2017) involved 795 Holstein heifer calves and found that heifers less than three months of age that had been diagnosed with respiratory disease were 10kg lighter than heifers that had not had an episode of respiratory disease. Furthermore, this difference in bodyweight became more divergent at fourteen months of age, rising to 29kg. Donovan et al. (1998) reported that respiratory disease slowed the growth of heifer calves within the first six months of life, such that they reached their target weight 13-15d (days) later than calves without disease. This exposure to respiratory disease can also have an impact on target weight at first service, thus leading to the animal being older than the industry desired target age of 24 months at first calving. Heinrichs et al. (2005) found there to be a tendency whereby calves that were treated with antibiotics for diarrhoea and pneumonia for a number of days had an increased age at first calving. Recent work by Boulton et al.

(2017) predicted that every day over this desired first calving age of 24 months would cost an additional £2.87 per day.

Bach (2011) examined the relationship between the number of episodes of BRD and that animal's survival in the milking herd. This work found that if an animal sustained more than four episodes of respiratory disease at any stage of development prior to first calving, then it was 1.87 times (± 0.14) as likely not to complete its first lactation, when compared to animals that had not experienced respiratory disease. A similar relationship was discovered by Waltner-Toews et al. (1986), who found that heifers that had been treated within the first three months of life were 2.5 times likely to die after ninety days of age. Schaffer et al. (2016) found similar results to the previous studies whereby dairy heifer calves that had respiratory disease at less than 120d of life had an increased risk of leaving the herd either before their first or second calving. The study by Schaffer et al. (2016) also highlighted that such animals were likely to have lower milk production in their first lactation.

1.1.3 Disease detection

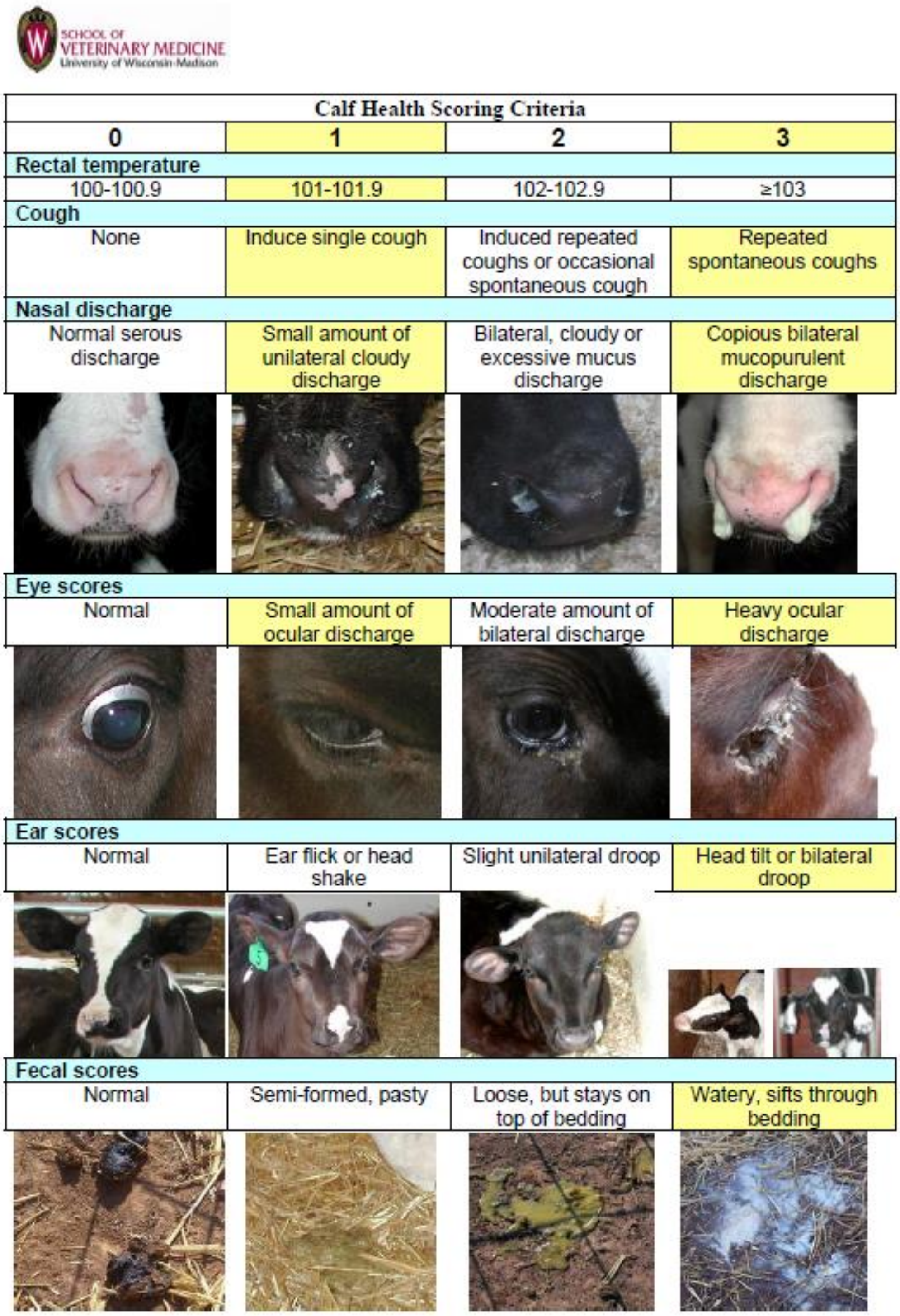
Having considered that BRD is an important disease in calves with major consequences for health and productivity, there is a need to reduce the impact of the disease on the calf, farm and cattle industry.

1.1.3.1 Disease detection rates

One of the first steps towards reducing the impact of the disease would be the implementation of detection methods. .An important factor in calf rearing is the ability of farmers/calf-rearers to detect signs of ill-health at an early stage of diagnosis to reduce the impact of disease on the calf and potential mortality. A retrospective study conducted by Olson et al. (2019) has shown the discrepancies between farmstaff and a veterinary observer in the treatment of calves. The study found that a clinical observation made by the veterinary observer was not always associated with the farmstaff's decision to treat the calves. In other words, the farmstaff were missing calves that were showing signs of disease. The farmstaff treated 390 calves out of 460 calves in total (84.7%) whereas the veterinary observer diagnosed 426 calves with abnormal clinical scores out of 460 (92.6%). The theme of poor detection amongst farmstaff is commonplace amongst numerous studies. Cramer et al. (2016) found that the sensitivity of farm disease detection was only 29%. Sivula (1996) found that cases of BRD were more likely to be missed by calf-rearers than misdiagnosed, with diagnosis being 56% sensitive but 100% specific. Therefore, it is evident that there is a need to develop techniques and technology to aid in calf disease detection.

1.1.3.2 Visual clinical scoring systems

The most common method used for the detection of disease in calves is via inspection by the personnel responsible on farm for rearing the calves, and is usually based on experience rather than following a set protocol. There are a couple of examples of ‘protocol based’ health scoring systems currently in use, mainly in research studies. There is no evidence to suggest that such scoring systems are being or have been used widely in commercial enterprises. The most widely used in research studies is the Wisconsin Health Scoring System (McGuirk, 2008) (Figure 1.1). This scoring system assesses the clinical signs of ocular discharge, nasal discharge, head and ear positioning, coughing (induced or spontaneous) and rectal temperature. These signs form the basis of a diagnosis of the presence of respiratory disease. These clinical signs are assigned a score from 0 to 3 based on the level of severity, with 0 regarded as normal and 3 being severe. Faecal consistency, navels and joints can also be assessed in this way by assigning a score between 0 and 3 based on severity. Using the Wisconsin scoring system, if a calf has a total respiratory score of five or greater or has two or three of the clinical signs with a score two or three, then it would be diagnosed as having BRD and should be treated accordingly (McGuirk and Peek, 2014).



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Calf Health Scoring Criteria			
0	**1**	**2**	**3**
Rectal temperature			
100-100.9	101-101.9	102-102.9	≥103
Cough			
None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge			
Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral, cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
Eye scores			
Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
Ear scores			
Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
Fecal scores			
Normal	Semi-formed, pasty	Loose, but stays on top of bedding	Watery, sifts through bedding

Figure 1.1 Wisconsin calf health scoring system (Source: University of Wisconsin (https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf))

Love et al. (2014) have also developed a simple scoring system, often referred to as the California Scoring system (Figure 1.2). This system considers similar clinical signs as mentioned for the Wisconsin health scoring system (ocular discharge, nasal discharge, head/ear positioning and cough) but also quality of breathing. However, the main difference between these two scoring systems is that the California system does not score each sign based on severity levels, but on whether or not sign is present or absent. Also, in terms of scoring cough, where the Wisconsin system includes inducing a cough, the California system is based on spontaneous coughs only. The California system is also based on weighted scores for each of the clinical signs. Using the California system, if a calf has a score of five or greater when the scores for the clinical signs are added together, then it would be regarded as being a BRD case (Aly et al., 2014).

Both of these scoring systems have been developed for intended use in pre-weaned dairy calves. Maier et al. (2019) created a scoring system for weaned dairy calves that is largely based on the same principles as the California scoring system (present/absent, weighted scores) and scores cough, abnormal respiration, body condition, sunken eyes and the range in ambient temperature over a 24h period.

They also concluded that adding rectal temperature to this scoring system increased the specificity but lowered the sensitivity. Overall this scoring system could be used as a tool to screen for BRD in weaned calves.

There is always going to be an argument as to which one of the health scoring systems is most accurate in detecting ill-health. A study by Aly et al. (2014) assessed one hundred calves using the Wisconsin and the California scoring systems. Within that study they found the Cohen's *k* between these scoring systems to be 0.85 which indicated that the scoring systems had excellent agreement. The sensitivity and specificity of both scoring systems was explored by Love et al. (2016), who established that the sensitivities of the Wisconsin and California scoring systems were not significantly different when used as either a screening tool or diagnostic tool (screening sensitivity *p=0.78*, diagnostic sensitivity, *p=0.70* for both scoring systems). However, there was a difference in the specificity between the two scoring systems (Wisconsin: 91.2%, California: 87.4%). Love et al. (2016) reflected on this difference by suggesting that it might be a more acceptable compromise based on practicality to use the California system, because less handling of the calf is required and it is therefore easier to use.

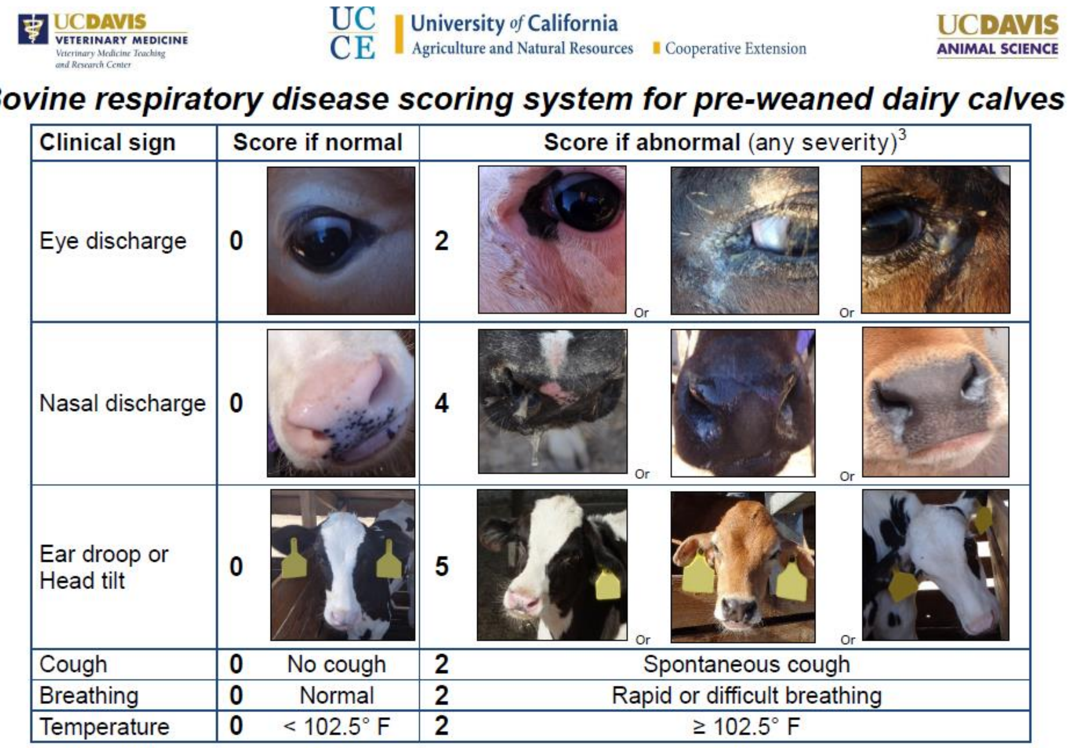


Figure 1.2 California calf BRD (Bovine Respiratory Disease) scoring (Source: University of California, Davis (https://www.vmtrc.ucdavis.edu/sites/g/files/dgvnsk5141/files/local_resources/pdfs/BRD%20scoring%20system%20chart%20logos%20v14.pdf))

The frequency at which clinical scoring systems should be carried out has also been investigated. A longitudinal cohort study conducted by Mahendran et al. (2017a) concluded that once weekly detection using such scoring systems was not sufficient. A review by McGuirk and Peek (2014) believed that performing the respiratory scoring twice weekly could improve the detection of respiratory disease amongst pre-weaned calves. Therefore a conclusion that can be drawn from both studies is that for calf health scoring systems to successfully detect signs of ill-health, they need to be carried out repeatedly to ensure no signs are missed. As McGuirk and Peek (2014) stated, the procedure of carrying out a health scoring system can mean additional work for staff, and they estimate that approximately an additional 0.5 FTE (full time equivalent) would be needed for every 100 calves. However, the authors do state that over time, this additional cost of labour will pay for itself in reduction in drug costs and calf mortality.

Buczinski et al. (2016) highlighted the differences between observers scoring the same set of calves and the influence this can have on the classification of the calf having or not having the disease. This can lead to an increase in false positive and false negative diagnosis of calves and the inappropriate treatment of cases.

1.1.3.3 Behaviour

The behaviour the calf expresses can be a sign of many factors, such as health status. A paper by Cramer et al. (2016) attempted to show that scoring the behaviour of calves could be used for detecting disease by using the measures of posture (standing and lying), isolation, approach tests and encouraging the calf to rise. Their study found that behaviour scoring had a sensitivity of 48.4% and a specificity of 79.4% for identifying severe BRD, with the severe BRD based on Wisconsin scores >6. The overall conclusion from the study was that this method should be used in conjunction with another detection method to improve detection rates. The duration of time at the water trough was one of the potential indicators of disease found by Lowe et al. (2019) along with duration of lying bouts. They showed that there was no difference in the number of times the calf visited the water trough either before or when clinical signs of disease were identified, but the calves spent more time at the water trough in the four days immediately before clinical identification of disease compared to the eight days before disease identification. When they compared the 4 days immediately before the clinical identification of the disease and the 4 days prior to this period, there was a decrease in the number of lying bouts but there was an increase in the duration of the bouts. Similar findings were found by Duthie et al. (2019). Their study also found that data from automatic milk feeders (number of visits to the milk feeder and time spent feeding from milk feeder) could be used for early indicators of disease amongst calves fed via such a feeding system.

Another behaviour that was found to be indicative of disease was the number of unrewarded visits to an automatic milk feeder (Svensson and Jensen, 2007). Other studies have also suggested that a combination of measures for detection would be advantageous (Sutherland et al., 2018). This study found that the use of data from automatic calf milk feeders and accelerometers to record lying behaviour both identified changes in behaviour in calves with calf diarrhoea. Similar conclusions were also drawn by Swartz et al. (2017).

1.1.3.4 Ultrasound

As well as the use of the visual clinical health scoring systems described previously, other methods have been used to specifically detect respiratory disease. One such method involves the use of ultrasound to detect consolidation of lung tissue. Rabeling et al. (1998) explored the feasibility of using ultrasound on calves with BRD. Their study found this technique to have a sensitivity and specificity of 85% and 98% respectively. They concluded that the technique could not only detect BRD, but also the severity level of it after evaluating parts of the lung structure from post mortem analysis. The amount of lung tissue affected by BRD would normally only be known at the point of post-mortem. Overall, the use of ultrasound would be a fast, accurate and above all, practical method for the detection of BRD in calves (Ollivett and Buczinski, 2016). Cramer and Ollivett (2019) looked into whether there was any benefit of adding the use of a scoring system such as the Wisconsin scoring system alongside ultrasound and found a good justification for such a combination.

1.1.3.5 Cough sound analysis

The analysis of coughing is a detection method that has been used in the pig industry, and there has been recent research into its use in the calf sector. Coughing can be an indication of respiratory disease. The analysis of coughing amongst pigs was carried out by Ferrari et al. (2008). They used an omnidirectional microphone and recorded coughs from infected pigs that had clinical signs of respiratory disease and compared them to induced coughs from known healthy pigs. Acoustic and visual spectral analysis was then used to analyse the cough sounds. They showed that a difference between the healthy cough and infected cough could be detected. Wang et al. (2019) examined whether or not the analysis of cough sounds could be used to assess the quality of the air within commercial pig housing. The results of their study showed that the algorithm that was developed to recognise different cough sound characteristics from other background sounds was 95% accurate. Their study also found that there was a difference in mean peak sound frequency of the cough sound samples between buildings that were either cleaned four times a day or twice a day, and therefore had a difference in their air quality in terms of respirable particles and ammonia levels.

Ferrari et al. (2010) considered the analysis of cough sounds in the detection of respiratory disease amongst dairy calves. This study found that it was possible to detect coughing from other sounds. A study by Vandermeulen et al. (2016) not only developed a calf cough monitoring algorithm, but compared it against the Wisconsin health scoring system, along with blood samples for blood neutrophil levels. A result of this study was that there was no relationship between the number of disease associated coughs and the Wisconsin health score obtained along with the results from the blood samples, even if the Wisconsin heath score and the blood results were detecting BRD. However, it might have been the case that calves with BRD did not display any clinical signs of a cough. Despite this, they suggest that the cough sound detection method could potentially be used as an early indicator of BRD, based on the method having 50.3% sensitivity, 99.2% specificity and 87.5% precision.

1.1.3.6 Thermal imaging

The reviews conducted by Sellier et al. (2014) and Godyń et al. (2019) show that the use of thermal imaging is starting to gain interest in the animal health sector. Many of the studies already carried out have been on adult cattle and not young pre-weaned calves. Some studies (for example, Schaefer et al., 2007, 2012) do refer to calves in their title. However, upon further investigation, they are referring to animals that are less than 9 months of age whereas this thesis considers the pre-weaned calf (i.e. much younger). Despite this interpretation of the term ‘calves’, both studies found that the use of thermal imaging was effective at detecting signs of BRD at an early stage in such animals. Thermal imaging will be discussed further in Chapter 2. Thermal imaging was considered for further study rather than other technologies due to it being non-invasive and that there has not been an extensive amount of research conducted in pre-weaned calves using thermal imaging.

1.2 Calf Housing

As mentioned previously, housing can have a causal role in BRD in terms of environment (air temperature, moisture, draughts, ventilation) and management decisions (stocking density, bedding type).

In the United Kingdom, dairy calves are mainly artificially reared and housed under a variety of systems, such as individual pens, individual hutches, group pens, group hutches or a combination of systems (for example, individual pen for the first 5 days of life and then introduced into a group-housing system). Many farms still adopt what could be classified, in terms of calf rearing, as the 'traditional system' of individual pens (occasionally within a redundant farm building that was for another purpose other than rearing calves) where the calf is bucket-fed as highlighted in a recent study by Boulton et al. (2015). This finding is in line with that found from a European study by Marcé et al. (2010) which included responses from the UK, who found that calves were housed after birth in individual pens in eleven of the fourteen countries questioned.

Despite all the various ways to house calves, the housing should provide the calf with the freedom from discomfort (DEFRA, 2003). The basic principles for calf housing should be to provide the calf with an environment that is clean, dry and free from draughts. The housing should also be well ventilated and provide a comfortable environment for the calf. Getting the calf environment correct is crucial in promoting health and maintaining growth rates (Webster, 1984).

1.2.1 Individual calf housing

The Council Directive 2008/119/EC states that no calf older than eight weeks of age can be housed individually. The exception to this is the calf can be housed individually if receiving veterinary treatment. Therefore, individual housing can only be a viable option for calves up to eight weeks of age.

The two options for individually housing calves are individual pens within a building and individual hutches. An individual hutch consists of a roofed, three sided enclosed structure that sometimes has a small 'outside' area that the calf can access. This housing practice is common in the United States (Stull and Reynolds, 2008).

These hutches can be manufactured from materials such as polyethylene or fibreglass that can be easily washed and disinfected between inhabitants. Another advantage of the individual hutch is that it can be placed anywhere around the rearing unit with a hard standing and does not need a dedicated overarching building. The hutch can provide protection for the calf therefore creating its own environment. A study by Macaulay et al. (1995) compared three types of individual housing comprising of wooden hutches, polymer hutches and polyethylene domes. They concluded that the polyethylene domes created the warmest microclimates.

Another benefit of housing a calf in a hutch is that it can avoid contact with other calves in the early stages of life. This is important as the calf has been born with an immature immune system (Hulbert and Moisá, 2016). A study in the United States by Wells et al. (1996) found that over 80% of calves were housed individually. Another US study compared individual housing types. Peña et al. (2016) compared a wire framed hutch with a sheet of plywood over the top of it that was placed under trees, and a completely enclosed polyethylene based plastic hutch that was out in the open. No difference was found between the two types of individual housing in terms of liveweight gain and there was a lower disease treatment rate in the plastic hutch compared to the wire framed hutch which would indicate from this study that there was an impact of hutch design and location on BRD occurrence.

The option of housing calves in individual pens within a building also offers the benefit of avoiding contact with other calves, but this is dependent on the structure of the pen. For example, an individual pen constructed from gates offer the ability for tactile contact with other calves in the adjacent pen(s), whereas using solid panels limit the opportunity for contact. The construction of individual pens in relation to the number of solid panels was part of an investigation by Lago et al*.* (2006) examining factors associated with respiratory disease. This study found that the prevalence of respiratory disease decreased with the presence of solid panels, and that the addition of solid panels at each end of the individual pen and/or a solid panel acting as a roof increased the bacterial count (cfu/m^3) in the air of the pen.

1.2.2 Group-housing

There is a growing trend for group rearing calves, whether this is from birth or after a few days of individual housing. However by group-housing calves there will be a trade-off between disease transmission and allowing calves to have social contact. As well as pressure to not keep calves in social isolation (DEFRA, 2003), a reason for group-housing calves has been the introduction of automatic feeders, which results in a reduction in the labour needed to feed calves. Reports estimate that the use of automatic feeders saves approximately 9 minutes per calf per day through not having to physically prepare the milk feed and then dispense it, as well as cleaning utensils afterwards (Kung et al., 1997). There would be some debate as to what constitutes a 'group', but literature suggests that housing calves in groups of no more than 10 is the ideal (Svensson and Liberg, 2006). Calves kept in more stable groups have better daily liveweight gain and lower incidences of respiratory disease and diarrhoea than those calves kept in dynamic groups (Pedersen et al., 2009). These results are reinforced by Wójcik *e*t al. (2013) who compared group penned calves within a building and calves kept in a group-housing igloo-style hutch, and also found similar results. There is also a growing trend towards the paired-rearing of calves. This is mainly due to milk processors recommending that farmers move more towards this system of housing calves based on consumer perceptions. As the next section highlights there are advantages and disadvantages to grouping calves as two calves could technically be regarded as a 'group'.

1.2.3 *Calf health and behaviour*

Each system of housing calves has its own advantages and disadvantages. Research by Chua et al. (2002) looked at the effects of paired-housing against individual housing in terms of calf health, performance and behaviour. They found that there were no negative effects of paired-housing calves from birth in terms of weight gain and health which was also found by De Paula Vieira et al. (2010). However (Chua et al. (2002) did state that the paired-housing system provided the calf with more space and allowed more movement as well as social contact with another calf. They reported that calves within the paired-housing system spent 2% of the day in social contact. Jensen et al. (1997) found that calves reared individually were more fearful of a novel social situation than calves reared in a group. Similar results were obtained from another study by Jensen and Larsen (2014) who also investigated the effects of social contact on calf health. In their study, Jensen and Larsen (2014) identified that pair-housed calves took less time to sniff an unfamiliar calf during a social test than calves that were individually housed. Also these calves, along with calves that were individually housed but had tactile contact with another calf, sniffed an unfamiliar calf for longer during social tests. This showed that calves that had contact with others were less fearful of new situations.

One reason regularly quoted for preferring individual housing for calves is the removal of social contact, and therefore inferring that individual housing will reduce the probability of a calf becoming infected from another. The study by Curtis et al. (2016) assumed that the increase in diarrhoea risk amongst calves came from group housing and that if calves in a group situation were fed via a single teat, for example the single teat of an automatic milk feeder, increased the risk of respiratory disease. However, recent research would suggest that the reversal of the perception that individually housing calves will reduce the probability of the calf becoming infected is more likely. The study by Jensen and Larsen (2014) showed that there was no effect of housing treatment on the levels of pathogens found in faeces or in the level of antibodies for the most common respiratory pathogens. In spite of this, a study from Norway examining mortality in dairy calves found that calves that had been housed in group pens were more likely to die within the first month of life compared to calves that were individually housed (Gulliksen et al., 2009). However, Hanninen et al. (2003) found that calves kept in groups had a lower incidence rate of diarrhoea during the first seven weeks of life compared to those calves that were individually housed. To conclude, it could be said that group-housing calves is better for social development, but with mixed results in terms of disease incidence.

Various research studies have shown an increase in calf performance when housed in group situations rather than individually. Bernal-Rigoli et al. (2012) found that calves that were group-housed were heavier by the end of their study than individually housed calves, but also that the group-housed calves were consuming more milk and solid feed by the end of the study. Tapki (2007) carried out an experiment whereby calves were housed individually for the first 33 days of life, and then some of these calves were housed in groups of three whilst the rest remained housed individually. They found that group-housed calves were 2.36kg heavier at the end of the study period than the individually housed calves ($p<0.05$), and ate more calf starter feed and alfalfa hay. Similar results were also found by Chua et al. (2002) who reported higher milk, starter and hay intake in group-housed calves compared to individually housed calves. However De Paula Vieira et al. (2010) reported in the experiment they conducted, that pair-housing did not influence weight gain, but they do mention that their results should be interpreted with caution. They found that calves that were paired-housed ate more starter pellets than individually housed calves during the pre-weaning phase, and infer that this result is a consequence of the social aspect of the calf housing and the paired calves learning from each other. Literature is starting to emerge that explores the cognitive performance of calves housed individually or in pairs/groups. Findings from Gaillard et al. (2014) and Meagher et al. (2015) show that individually housed calves had learning deficits compared to pair-housed calves.

1.3 Air quality and thermal environment of calf housing

As previously mentioned in section 1.2, regardless of housing style and method, the housing should provide the calf with an environment that is well ventilated but draught free and provide thermal comfort. However, there is a fine balance between creating a comfortable environment for the calf and one that is not. An uncomfortable environment for the calf can be created as a result of the farmer/calf-rearer feeling uncomfortable themselves. It is worth remembering that on the majority of occasions, the calf cannot physically remove itself from an uncomfortable environment within its housing due to the restrictions imposed by the physical housing environment.

1.3.1 Air quality and ventilation

One aspect for creating a comfortable environment for the calf is the quality and hygiene of the air in the calf housing. This can be achieved through adequate ventilation. Ventilation is a major risk factor for BRD in calves as inadequate ventilation is often associated with poor calf health (Lorenz et al., 2011). The main role of ventilation is the exchange of air by the removal of products produced by the calves (heat, moisture, carbon dioxide and other gases) along with dust and airborne micro-organisms and replacing this stale air with a supply of fresh air (Wathes et al., 1983). Calf housing can be ventilated either by natural ventilation or through mechanical ventilation (e.g. positive pressure tube systems and negative pressure systems). Naturally ventilated calf houses rely on the natural forces of cross draughts and the stack effect (Chamberlain, 2015). Cross-draughts tend to be the main source of airflow in naturally ventilated calf housing as most buildings are situated to take advantage of the prevailing winds. A study carried out by Lago et al. (2006) found that wind was the major drive for ventilation on the farms that were involved in their study. One issue with the reliance on cross-draughts in ventilating a building is the variability in providing fresh air and it is also uncontrollable. A recommendation made by Webster (1984) is that wind speed should be less than 0.25m/s at calf level with anything above this being classified as a draught. Therefore it could be said that there is a slight trade-off between ensuring that there is enough fresh air coming into the calf housing but at the same time making sure that there is not too much fresh air coming into the building that it creates a draught at calf level. However, to prevent draughts being created, the fresh air inlets should be above the height of the calves (Teagasc, 2017).

The movement of air through a calf house by the stack effect relies on the air being warmed by the calf sufficiently to create an upwards draught that contains the contaminated air. (Figure 1.3). This contaminated air can be removed from the building via the ridges of the roof, and is replaced with fresh air via side inlets. Nordlund (2014) states that for the stack effect to occur, there needs to be a $2^{o}C$ difference between the inside temperature and outside temperature to drive it. Also, Nordlund (2014) suggests that the stack effect will not always be found in calf houses. This is because the body temperature of calves is not sufficient to result in a temperature gradient to stimulate the stack effect. Anecdotal evidence would suggest that the stack effect does not function in the majority of traditional calf housing.

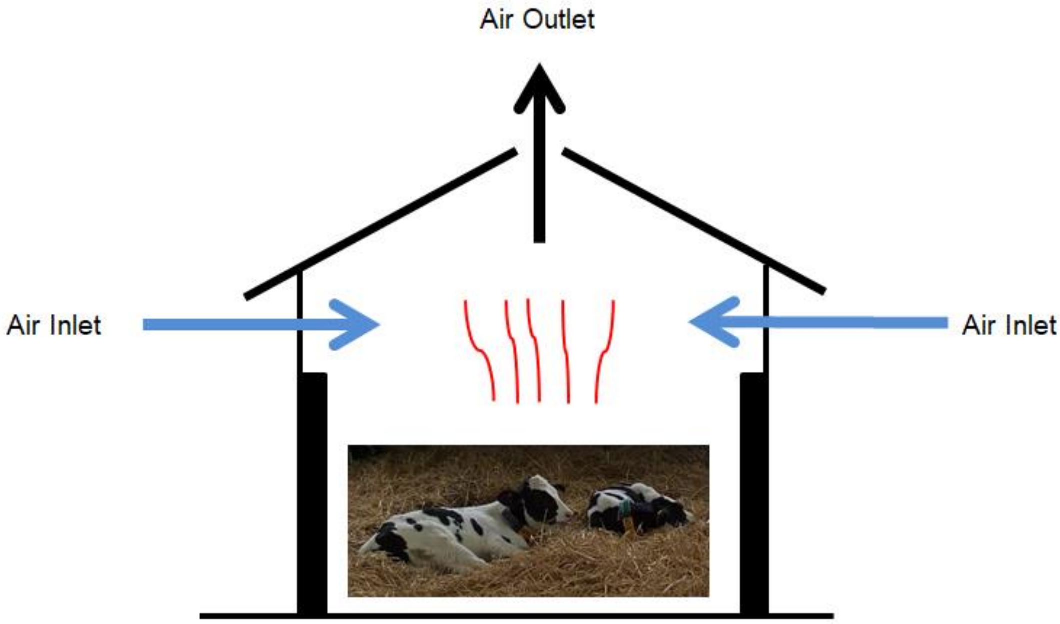


Figure 1.3 Illustration of stack effect

1.3.2 Thermo-neutrality and thermal comfort

The vast majority of the work mentioned in this review of the literature fails to highlight the time of year that the work was conducted and the thermal conditions, for example air temperature that the calves experienced during the studies. Therefore it is unknown if the calves in the various studies were kept in housing conditions that implemented thermal neutrality. In many circumstances, a calf born in the UK will be exposed to environmental temperatures that do not meet its thermal neutral zone (TNZ); therefore creating a less than optimum environment that may affect its growth and ability to resist disease. It is therefore important to understand the principles of thermal neutrality and how to provide this for calves.

The neonatal calf is born into an environmental temperature that is significantly lower than the temperature *in utero* (Vermorel et al., 1983; Rowan, 1992; Carstens, 1994). As it is a homeotherm, the calf needs to maintain a constant core body temperature. To achieve this, it must try and balance the amount of heat produced with the amount of heat it loses to the environment. Bligh and Johnson (1973) define the thermal neutral zone (TNZ) as, "*the range of ambient temperature within which metabolic rate is at a minimum, and within which temperature regulation is achieved by non-evaporative physical processes alone*". The TNZ can be thought of as having three zones within it: cool, thermal comfort and warm (Figure 1.4).

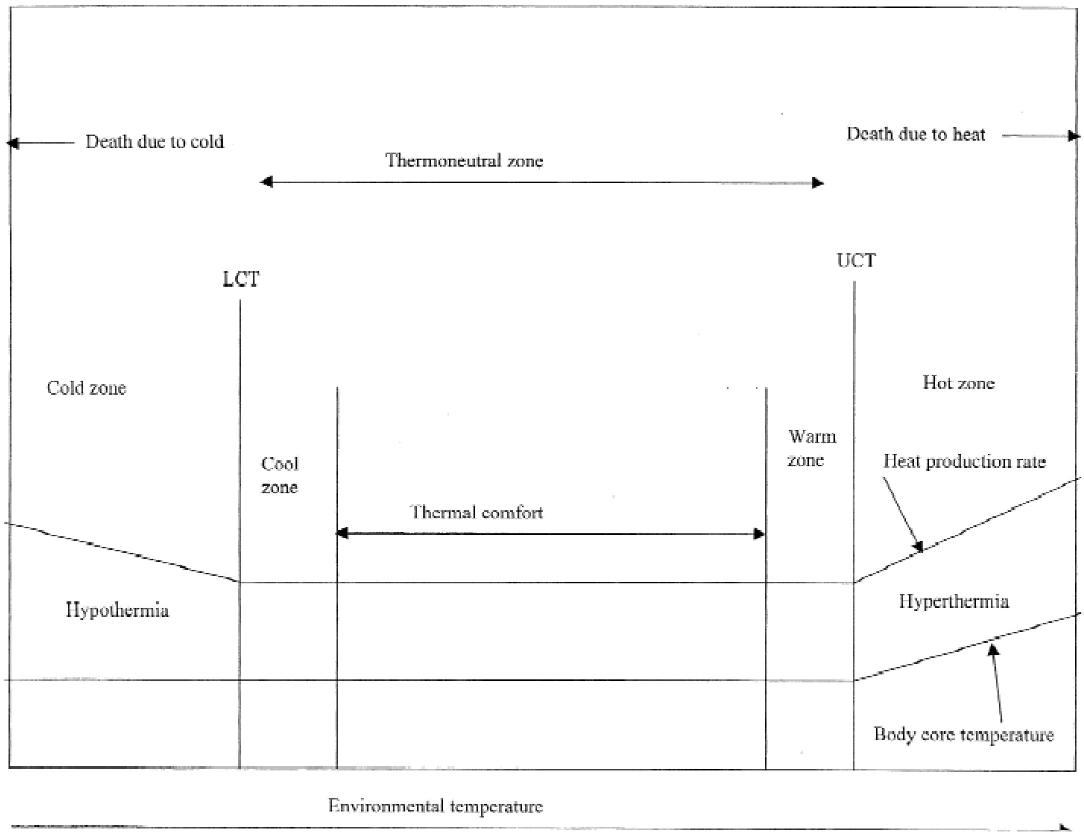


Figure 1.4 Illustration of thermal zones (source: Kadzere et al., 2002**)**

The thermal comfort zone (TCZ) is the environmental temperature at which the animal (in this case the calf) is not motivated to perform any thermoregulatory behaviour. Kingma et al*.* (2014) alludes to this when they mention that the ranges of ambient temperatures associated with the thermal comfort zone are smaller than that of the thermal-neutral zone. As the environmental temperature reduces, the calf then starts to enter a cool zone.

Within this cool zone, the calf still does not use any additional energy to produce heat to maintain warmth, but instead the non-evaporative processes such as behavioural changes are used. In other words, the calf will use the behaviour it expresses as a mechanism to signal how it perceives its environment, for example, removing itself from a specific environment (Baldwin, 1973). Therefore, the calf will be using its behaviour to try and mitigate its perception of hot or cold. Gradually within this cool zone, as the environmental temperature decreases, it reaches the lower limits of the TNZ. This point is referred to as the Lower Critical Temperature (LCT). Similar mechanisms apply when the environmental temperatures start to increase from that out-with the TCZ. The calf experiences a warm zone, with the upper limit of this warm zone being referred to as the Upper Critical Temperature (UCT).

For calves, the TCZ is estimated to be in the range of 15 to 25^{o}C according to Stull and Reynolds (2008). The TNZ is not a fixed range of environmental temperatures due to the influence of wind speed, humidity levels, the physical age of the calf and also the plane of nutrition on the ability of the calf to maintain core body temperature (Roland et al., 2016). The influence of wind speed, moisture and age of the calf is described in section 1.3.3, and the plane of nutrition is described in section 1.3.6.2.

1.3.3 Lower Critical Temperature (LCT)

Environmental temperatures in the UK will not always be ideal for calves. The most recent summary data available from the Met Office (from 1981 to 2010) has the mean annual maximum temperature of 12.4^{o}C and a mean annual minimum temperature of 5.3^{o}C (Met Office, 2020). At present, it is more common for air temperatures to be below that of the temperature range of thermal comfort mentioned by Stull and Reynolds (2008) than above it.

As previously mentioned, the LCT is the environmental temperature at the lower end of the thermo-neutral zone, and is the stage at which the calf needs to start producing more metabolic heat to maintain thermal balance. This can be done by contraction of the skeletal muscles (shivering) or through non-thermogenic processes such as increasing energy intake. Similar to the TNZ, the LCT is not a fixed environmental temperature, and is influenced by air speed, humidity levels, nutrition and age of the calf. Gonzalez-Jimenez and Blaxter (1962) carried out a series of experiments to try and determine the environmental temperature at which heat production is increased as a result of increasing cold. A general finding from their study was the LCT for calves fed four litres of milk per day reduced over time from 12.8^{o}C at day 3 of life to 8.2^{o}C at day 20 of life. This evidence would suggest that as the calf grows older, the requirement to produce heat to maintain core body temperature was less. One of their overall recommendations was that the general environmental temperature within a calf house should not fall below 13^{o}C. Work by Holmes and Mclean (1975) showed that there was an effect of wind speed on the amount of heat produced. Calves exposed to wind needed to produce more heat than when they were not exposed.

As Table 1.2 displays, as air speed increases, the LCT of the calf, regardless whether it is a new-born or older calf, increases too. This is as a result of an increasing air movement dislodging the external insulation that the hair coat provides the calf (Webster, 1974).

Table 1.2 Effect of air speed on lower critical temperature (LCT) (Webster, 1981)

	Lower critical temperature (^{o}C) at air speeds of:	
	0.2m/s (draught free)	*2.0m/s*
Newborn (35kg)	+9	+17
One month (50kg)	0	+9

Table 1.2 and the work by Gonzalez-Jimenez and Blaxter (1962) also show that the age of the calf can affect the LCT. As the work by Gonzalez-Jimenez and Blaxter (1962) showed, the reason behind this is that as the calf gets older it does not need to produce as much heat to maintain warmth.

Webster (1984) highlight the importance of floor type and bedding on the LCT of calves in that a dry concrete floor is extremely cold and can raise the LCT of a newborn calf to 18^{o}C. It is also mentioned that the LCT is reduced when dry straw is used as bedding compared to damp straw.

1.3.4 Heat stress

Although not commonly regarded as an issue for farmers in the UK, high temperatures are another source of thermal stress. However, with the phenomenon of global warming, air temperatures are constantly increasing (approximately by 1.53^{o}C) (IPCC, 2019) and therefore high temperatures (and subsequently heat stress) may become another aspect of the calf housing environment that will need to be taken into consideration in future years in the UK. Environmental temperature in excess of the TCZ (i.e. in excess of 25^{o}C) is referred to as Upper Critical Temperature (UCT). Kadzere et al. (2002) makes reference to the UCT being the environmental temperature at which the animal needs to reduce heat production as a result of an increased core body temperature and therefore the animal could be described as being heat stressed. A study conducted by Broucek et al. (2009) found that calves that were born in periods of high temperature were likely to have low daily liveweight gain (DLWG), low intake of starter concentrate and a high consumption of water from birth until weaning. This was probably due to the calves trying to dissipate the heat from the environment and therefore using energy to do so.

1.3.5 Interpretation and guidance

Reviewing the literature has shown differing uses of the terminology to describe thermal comfort and neutrality, with the main confusion surrounding the interchanging of such terms. For example, Davis and Drackley (1998) state that "*All warm blooded animals (homeotherms) exhibit a thermal comfort zone, which is more frequently referred to as the zone of* *thermoneutrality".* In this definition, the TCZ is equivalent to the TNZ, which is at odds to how these terms are defined by Bligh and Johnson (1973) and Yousef (1985).

There is also some variation in the scientific literature as to the range of environmental temperatures that are considered the TNZ, TCZ and LCT. Davis and Drackley (1998) declare that based on their explanation of TNZ, the TNZ is environmental temperatures between 10 and 15^oC but further on in their report, they highlight that a study by Gebremedhin et al. (1981) claims that the TNZ is between 15-25^oC. Stull and Reynolds (2008) also claim that the TNZ is between 15-25^oC. A summary of TNZ of some ungulates by Yousef (1985) suggested that the TNZ for calves to be 13-25^oC. There is also a lack of clarity in statements about the LCT.

A review of LCT by Davis and Drackley (1998) quoted that Gonzalez-Jimenez and Blaxter (1962) considered the LCT of calves between 4 and 23 days of age to be 8.3^oC, whereas Schrama et al. (1993) believed that the LCT for calves between 6 and 14 days of age was between 12.5 and 14.5^oC. The study by Gebremedhin et al. (1981) described that the LCT for calves was somewhere between 10-15^oC. Webster (1974) predicted that the LCT for a newborn calf was 9^oC and 0^oC at one month old.

A review of information freely accessible to farmers from nutritional companies and advisory agencies also highlights the variation in advice that is currently available to UK farmers (Table 1.3).

Some of this variation, especially from commercial companies, will be as a result of updating information as it is made available. However, no reference to the source of information is given in these publications.

What the reviews of both the scientific literature and the advice that is freely available to farmers show is that the TNZ, TCZ and LCT are not static, and that individual circumstances should be considered, e.g. age of calf, wind speed, plane of nutrition etc. Unfortunately what also emerges is that there is confusion over the interpretation of the principles of thermal-neutrality as alluded to by Yousef (1985). Overall, more clarity over the basic fundamentals is needed, and identifies not so much as a 'gap' in the knowledge, but more of understanding and application of the existing knowledge.

Table 1.3 Current advice given to farmers in the UK on environmental temperatures for calves

Recommendation/Advice	Source
Regardless of housing type, a newborn calf needs to be kept in a temperature not less than 7^{o}C. By one month, calves can comfortably withstand temperatures around freezing point.	DairyCo (2012
In the 1st week of life, calves start using energy from feed to keep warm at temperatures below 15^{o}C. By the 4th week, calves will not feel cold until about 0^{o}C	Volac (2015)
Newborn calf needs to be kept in temperatures no less than 7^{o}C. By one month of age, calves can withstand temperatures around freezing (0^{o}C)	NADIS (2016)
A comfortable microclimate must be provided in the first week of life with temperatures > 20^{o}C	Teagasc (2017)
Calves have the potential to suffer from cold stress during the winter when temperatures drop below 7^{o}C	Wynnstay Agriculture (2017)
The Thermo neutral zone is between 10-20^{o}C. For calves less than 3 weeks, the LCT is 20^{o}C and for calves greater than 3 weeks, the LCT is 10^{o}C	ForFarmers (2018)
Newborn calves feel cold when temperatures are between 10-15^{o}C. By one month of age, a healthy calf can comfortably withstand temperatures around freezing	AHDB (2018a)
Newborn calves feel cold when temperatures are between 10-15^{o}C. By one month of age, a healthy calf can comfortably withstand temperatures around 6-10^{o}C	AHDB (2018b)
Cold stress becomes an issue for most calves when temperatures reach below 10^{o}C, or lower critical temperature. Calves less than three weeks of age have a lower critical temperature of just 20^{o}C	ForFarmers (2019)
The LCT for calves less than 3 weeks of age is 10-15 degrees and is highly dependent on air speed. The LCT for calves over 3 weeks of age is between 6-10 degrees and is highly dependent on air speed	AHDB (2020)

1.3.6 Practical adaption strategies

There are a number of housing and management strategies that farmers can use to mitigate the risk of cold stress in calves.

1.3.6.1 Provision of bedding material and nesting

One of these mechanisms is to encourage nesting behaviour in calves. Nesting behaviour is when the calf tries to bury itself within the bedding material, hence creating a nest-like feature. Nesting scoring can be carried out as a quantification of thermal comfort (Figure 1.5) with an example of its use in research being Lago et al. (2006). The question of quantity of bedding to use for calves has not been extensively examined. One reason for this could be due to the variations in bedding materials used on a global scale (e.g. in Canada, sawdust is used, whereas in the United Kingdom and Ireland, it is predominantly straw used to bed calves). There can also be considerable variation in quality between straws used for bedding. Teagasc (2017) make the recommendation that 20kg of bedding material per week should be used per calf at a depth of 15cm or more. However, there is no indication of how this figure has been derived and also under which environmental circumstances it was derived.

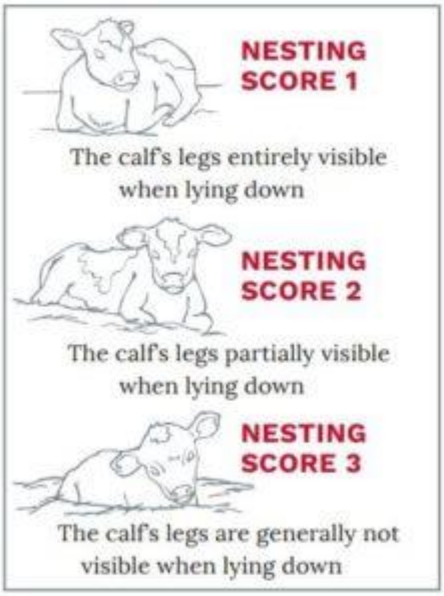


Figure 1.5 Example of calf nesting scores (Source: CalfCare (2019))

1.3.6.2 Feeding rates

During environmental temperatures that are below the LCT, calves need to increase heat production. To alleviate the effects of low environmental temperatures on the calf, feeding levels, such as the energy content can be increased during such periods. Schrama et al. (1993) carried out a study where they fed a constant energy level of milk to calves under four different environmental temperatures. They found that at low environmental temperatures of 5^{o}C and 9^{o}C, the calves produced more heat than they did at 13^{o}C and 18^{o}C, and subsequently their energy retention was lowered and use of body reserves increased. Early work by Gonzalez-Jimenez and Blaxter (1962) compared the heat production from calves fed four litres or six litres of milk. They showed that the resistance to cooling in the calves was less in those fed the six litres than those fed the four litres. Another finding from that study was that at three to four weeks of age, the LCT of the calves fed the six litres of milk was 7-8^{o}C, compared to 10^{o}C in the calves fed four litres. A study by Scibilia et al. (1987) discovered that increasing the energy density of the milk diet in terms of increasing the fat content caused an increase in weight gain. When the calves used in the study were kept at -4^{o}C and fed a fat content of 10%, they could not sustain their bodyweight, whereas when calves were housed at 10°C and fed the same level of fat content, they were able to gain weight. Table 1.4 is based on recommendations by National Research Council (2001) highlighting the increase in energy that the calf requires at different ages and different environmental temperatures.

1.3.6.3 Calf jackets

Another way to attempt to maintain thermal neutrality and comfort in calves is through the application of calf jackets to reduce heat loss to the environment. The use of calf jackets is increasing in popularity, with some research into their use now starting to emerge. Evidence to suggest that calf jackets do improve the temperature of the calf comes from the work of Scoley et al. (2017). Calves wearing calf jackets had a mean skin temperature of 35.5°C whereas calves not wearing calf jackets had a mean skin temperature of 29.1°C when they were housed in ambient temperatures of 7.7°C (±3.82). Conversely, Earley et al. (2004) published that there was no beneficial effect of using calf jackets on calf performance between calves kept outdoors (average temperature 4°C) and calves kept inside (average temperature 6°C). Bleach et al. (2016) examined the timing of the removal of calf jackets in terms of growth and health. They found that there was no significant difference in growth, health or concentrate intake on calves that had calf jackets removed at 3 weeks compared to 6 weeks of age. The differences in results suggest the need for more research into their effectiveness at different air temperatures, and also the age of the calf on which they would be most benefit.

1.3.6.4 External heat sources

The use of external heat sources such as infrared lamps, are a strategy that could be adopted to mitigate the risk of cold stress and provide calves with a comfortable microclimate (Bhat et al., 2015; 2017). Both of these studies have also shown the benefit of external heat sources in terms of daily liveweight gain. Bhat et al. (2015) showed that calves reared from birth until nine weeks of age with infrared lamps gained nearly 90g/d more ($p<0.01$) than calves reared without any additional heat source and Bhat et al. (2017) reported a gain of nearly 100g/d in the calves reared with the infrared lamps. Another interesting result from both of these studies was that at day 45 of age, the group of calves reared without the infrared lamps had significantly increased levels of cortisol in their blood ($p<0.05$). The relevance of this finding is that the hormone cortisol is used to quantify the response to stress. Therefore in both of these studies, the group of calves reared without the additional heat provided by the infrared lamp have shown signs of stress from their environment.

Table 1.4 Effect of environmental temperature on energy requirement of calves (source: National Research Council 2001)

Environmental temperature (^oC)	Increase in Maintenance Energy requirement (kcal of NE_M/day)		Maintenance Energy requirement (kcal of ME/day)		Percentage Increase in ME required for Maintenance	
	Birth to 3 wk	>3 wk	Birth to 3 wk	>3 wk	Birth to 3 wk	>3 wk
20	0	0	1735	1735	0	0
15	187	0	1969	1735	13	0
10	373	0	2203	1735	27	0
5	560	187	2437	1969	40	13
0	746	373	2671	2205	54	27
-5	933	568	2905	2437	68	40
-10	1119	746	3139	2671	86	54
-15	1306	933	3373	2905	94	68
-20	1492	1119	3607	3139	108	81
-25	1679	1306	3834	3373	121	94
-30	1865	1492	4066	3607	134	107

Notes: calculated for calf weighing 45.35kg. Extra heat production = 2.15kcal/kg$^{0.75}$ per day for each degree in environmental temperature (^oC) below lower critical temperature. Heat production is in terms of net energy (NE), Metabolisable energy (ME) was computed as ME = NE/0.8

[a] *Maintenance energy requirement 100kcal/kg$^{0.75}$ per day*

[b] *Calves from birth to 3wk have lower critical temperature in range of 15-25^oC, data calculated on basis of lower critical temperature 20^oC*

[c] *Data for calves >3wk were calculated on basis of lower critical temperature 10^oC*

1.4 Conclusions

Respiratory disease in calves is a challenging problem due to the complexity of its multifactorial nature. The housing environment is an important area where this disease can be prevented or at the very least reduce the risk of the disease by ensuring that thermal stress of the calf is minimised. Ideally, calves should be reared in their TNZ to minimise the effect of the thermal environment from a welfare and performance perspective, but this is not possible on many occasions due to the variability of the climatic conditions in the UK. It is also vital to ensure adequate ventilation of calf housing so that moisture, hot air and airborne pathogens can be removed from the calf environment. As has already been mentioned, one of the fundamental methods for this is to cease the initial generation of such fomites. However, there is a major trade-off between providing enough fresh air and not creating draughts.

As it has been highlighted in previous sections, BRD has huge financial implications, not only on calf producers but for the UK cattle industry as a whole. Producers seem aware of some of the causal factors for BRD, yet BRD remains a common disease on cattle farms worldwide. It is felt that a lot of emphasis has been placed on improvements to ventilation by producers; however other stressors and associated management decisions surrounding calf housing are often over looked and some simple changes can reduce the number of calves being treated for BRD. The main route of treatment of BRD is with antibiotics (Teixeira et al., 2017), with Sawant et al. (2005) reporting that antibiotic usage for calfhood diseases was largest for calves with enteric diseases (36%) followed by calves with respiratory disease (25%). With growing concern over antibiotic resistance, assurance schemes such as Red Tractor are monitoring antimicrobial usage on farms with the aim of reducing their usage (Hyde et al, 2019).

Overall, it has been highlighted that the housing environment of calves to prevent respiratory disease can be challenging. The work in this thesis focusses mainly on the housing environment of calves, as this is an area which has not been investigated to any extent.

1.4.1 Aims and objectives

The overall aim of this thesis was to improve the health, welfare and general performance of pre-weaned calves by investigating the effect of the environmental stressors that are risk factors of BRD (air temperature, wind speed, air quality) on the behavioural reactions and performance of pre-weaned calves. By understanding the effects of these environmental stressors better and taking remedial action to combat them, then there is the potential to use less antibiotics and achieve target weight gains as well as fulfil lifetime production. Another aim of this thesis was to develop a better practice for assessing core body temperature through the use of a non-invasive technology as such a technology has the potential to reduce stress associated with handling.

There are three main objectives for this study. As part of this work, a non-invasive method for core body temperature acquisition in calves (thermal imaging) will be explored (Chapter 2). The review of the literature has shown that there is some emerging work on calves involving the use of thermal imaging. However, most of this work has not focussed on the relationship between core body temperature and the temperature obtained from a thermal image or how this relationship could be improved by the inclusion of other parameters such as air temperature, relative humidity and wind speed. An elevation in core body temperature is one of the clinical signs of BRD. The current practice for obtaining core body temperature is by rectal temperature capture which is invasive and also involves the handling of the calf. The hypothesis was that thermal imaging could be used as a proxy for monitoring calf body temperature, and so developed as a method of detecting BRD.

Within the calf housing, there can be microclimates that can cause the calf to display a response such as removing itself from the hostile environment. The calf is also exposed to naturally occurring variations in environmental conditions on a daily, and occasionally hourly, basis during this vulnerable period of life and experience a chill or feel cold. The next objective was to consider the effects of climatic conditions on the behavioural response of the pre-weaned calf (Chapter 3) as well as the performance of the calf in terms of daily liveweight gain as a measure of growth (Chapter 4). The literature has illustrated the behavioural reaction of calves to the environment but it has not challenged if the calf responds differently under varying levels of the same parameter such as air temperature and wind speed. Also, the studies conducted to examine the daily liveweight gain of calves have mainly been carried out under controlled conditions, whereas in reality, the calf is exposed to varying environmental conditions. The calf house can be a thermal dynamic place and therefore any environmental parameter used should reflect this. Therefore, in Chapter 4 effective temperature was used as this incorporated air temperature, wind speed and relative humidity. The hypothesis was that calves exposed to adverse environmental conditions (temperature and wind) would seek to remove themselves to a better environment and that exposure to such adverse environmental conditions would result in lower daily liveweight gain.

An important physical aspect of calf housing is ventilation as it allows the removal of airborne microorganisms, particulate matter and moisture from the air. In the first instance, the best method is to prevent the generation of such fomites and therefore the third objective of this study was to examine the effect of a specific management decision, such as the provision of bedding material of varying quality, on the quality of the air in calf housing (Chapter 5). The hypothesis was that poorer quality bedding material would release more particles into the air increasing the potential risk of BRD. The review of the literature has shown that there is currently limited information on the quality of air within calf housing. Also, the studies that have been carried out have been conducted on commercial farms with varying management practices. Therefore, there has not been any controlled study carried out focussing directly on factors such as the effect that the presence of bedding material, the quality of the bedding material and the presence of calves has on the quality of the air in terms of particulate matter and bacteria.

Chapter 2: Comparison of thermal imaging and rectal temperature in the diagnosis of pyrexia in pre-weaned calves using on farm conditions

A manuscript based upon this chapter has been published (see Appendix C)

D.J. Bell, A.I. Macrae, M.A. Mitchell, C.S. Mason, A. Jennings, M.J. Haskell (2020) Comparison of thermal imaging and rectal temperature in the diagnosis of pyrexia in pre-weaned calves using on farm conditions. Research in Veterinary Science, Volume 131. Pages 259-265.
https://doi.org/10.1016/j.rvsc.2020.05.004

2.1 Introduction

Diarrhoea and respiratory disease are major causes of mortality in artificially reared pre-weaned calves. Early detection of these diseases can result in earlier treatment, thereby reducing their severity for the calf and potentially reducing spread to other members of the group. Body temperature is used as an aid in the diagnosis of ill health in calves (McGuirk and Peek, 2014). Changes in core body temperature can be an early indicator of disease, being one of the first clinical signs to manifest in cases of respiratory disease (McGuirk and Peek, 2014). Ill-health in calves can be identified more often using body temperature measurements than visual observations alone. The body temperature of the animal will rise as a sign that the immune system of the animal is fighting against a disease-causing organism. The most common method for taking body temperature in calves is by inserting a thermometer into the rectum of the animal. This technique requires restraint of the animal, which in itself can act as a stressor. In addition, this method has no agreed technique (Naylor et al., 2012) and is not without errors. One possible source of variation that could cause error is the presence or absence of faeces in the rectum at the time of measurement. As Burfeind et al. (2010) showed, the depth of insertion of the thermometer into the rectum can influence the result gained. Their study found that the temperature obtained at an insertion depth of 11.5cm was up to 0.4^{o}C higher compared to temperatures obtained from an insertion depth of 6cm. The procedure of taking rectal temperature is time-consuming and disruptive to the animal due to its invasive nature, and potentially inaccurate. For these reasons, other techniques to measure core body temperature more easily need to be explored.

A number of novel non-invasive technologies have been developed. Rumen temperature boluses have been developed to record body temperature in cattle (Knauer et al., 2016). However, such a device would still require an invasive procedure (oral administration) to be carried out to enable temperature to be recorded. Also such a device requires a developed rumen and as such would not be of use in newborn calves. However, there has been a temperature bolus very recently developed for calves (FarmFit™, *STgenetics, 22575 State HWY 6 South Navasota, TX 77868*) where the bolus resides in the abomasum until the rumen starts to develop. FeverTags™ (*FeverTags, 3846 Business Park Drive, Amarillo, Texas 79110, USA*) have been used to assess body temperature of young and growing classes of bovines in studies by McCorkell et al. (2014) and Mahendran et al. (2017b). This technology involves the fixing of the device to the ear and the insertion of a probe into the ear canal to allow for continuous monitoring of body temperature. However, McCorkell et al. (2014) stated that these tags did not always detect sick animals. Suggested reasons for this were the placement of the tag in the ear, as well as the displacement of the ear canal probe. If the tag was placed more laterally in the pinna of the ear, then this reduced the depth of the probe in the ear canal.

A contactless, non-invasive technology that is gaining recognition and momentum in the field is infrared imaging, also referred to as thermal imaging. Thermal imaging devices detect radiated energy from objects, and create electronic images based on how much radiated energy is emitted. The hotter the object is, the more radiated energy is emitted.

This non-invasive technology can be used for assessment of the health and welfare of cattle (Stewart et al., 2005; Theurer et al., 2013). For example, the technique has been used for the detection of lameness (Nikkhah et al., 2005; Alsaaod and Büscher, 2012; Alsaaod et al., 2014) and mastitis in dairy cattle (Berry et al., 2003; Colak et al., 2008; Hovinen et al., 2008) as well as to detect early signs of bovine respiratory disease in growing cattle in feedlots (Schaefer et al., 2007, 2012).

There is an emerging body of research where the use of thermal imaging in pre-weaned calves has been investigated. Stewart et al. (2008a) used this technique to examine the response of calves to disbudding, and Lowe et al. (2016) have used thermal imaging for the early diagnosis of calf diarrhoea.

Scoley et al. (2018) examined the variability and repeatability of thermal imaging in young calves.

The aims of this study were to determine if thermal imaging could be used as an alternative method to assess core body temperature in pre-weaned calves. The correlation between core body temperature (assessed using a rectal thermometer measurement) and the temperature obtained via a thermal image of the medial canthus of the eye was investigated. In addition, a multivariable predictive model was developed that incorporated climatic variables with the aim of improving the ability of the thermal imaging measurements to predict rectal temperature as a proxy for core body temperature.

2.2 Materials and methods

2.2.1 Calves – housing and diet

The experiment was conducted at SRUC Dairy Research & Innovation Centre, Crichton Royal Farm, Dumfries between 9 January 2017 and 3 September 2017 (Home Office PPL P204B097E). The study used 100 pure-bred Holstein male and 25 pure-bred Holstein female calves born from the dairy herds at the Centre.

2.2.2 Pre-Trial (individual hutch)

All calves were removed from their dam within 24 hours of birth, identified by the insertion of eartags; navel dipped with iodine, and fed four litres of thawed high quality colostrum. Once removed from their dam, the calves were transferred to the calf rearing building and placed in an individual calf hutch (*Calf-Tel, Hammel Corporation, Wisconsin, USA*) that was bedded with straw. Female calves were fed in accordance with normal farm practice which was three litres of reconstituted milk replacer (*Omega Gold*, *ForFarmers, Suffolk, England*; crude protein 23%, crude fat 18%, crude fibre 0.1%, crude ash 8.5%) at a concentration rate of 15% fed twice a day (0730h and 1600h) from teat feeder buckets. As part of another research trial, if the male calves still appeared to be hungry (i.e. investigating or licking the teat bucket) after their initial three litres of milk, then they would be offered an additional three litres. This practice was repeated at both feeds during the day. Whilst in the individual hutches, all calves, regardless of sex, had ad-libitum access to fresh water and starter pellets (VitaStart + Deccox, *ForFarmers, Suffolk, England*; crude protein 18%, crude fats & oils 4%, crude fibre 11.5%, crude ash 7%, 3mm diameter) both of which were fed via two small buckets. All feeding buckets (i.e. water bucket, milk teat feeder bucket, concentrate pellet bucket) remained with each calf for the duration of time that each calf was in the individual hutch. They were then removed and washed before being used for another calf. The individual hutch was also washed between calves and the bedding removed.

2.2.3 Trial (group hutch)

Both male and female calves remained in the individual hutches for seven days. They were then eligible to be moved to one of the eight group hutches using the Igloo system (*Holm & Laue, Westerfeld, Germany*). The Igloo system consisted of a hand laminated fibre-glass constructed dome (igloo) (3.9m, 4.4m, 2.2m) which provided the calves with shelter. In front of the Igloo there was a roofed pen (5.1m x 5.1m – length x width). Both areas were covered in straw, and this was replenished on a weekly basis as well as regularly removed (every 2 weeks). Calves were fed milk via an H&L 100 automatic milk feeder (*Holm & Laue, Westerfield, Germany*). Once in the group pen, male calves were allowed to drink up to eight litres of reconstituted milk replacer every 12 hours, resulting in a maximum allowance of 16l/d until the commencement of the weaning process at day 40 of age. There was no restriction on volume per visit to the automatic feeder, thereby allowing the male calves to consume all eight litres in one visit. Female calves had access to 3.6l every 12h, resulting in a maximum allowance of 7.2l/d until day 40 of age, when the weaning process began. However, at every visit to the feeder, female calves were restricted to 1l per visit. Apart from maximum volume and volume per visit, there were no other differences in the milk feeding process for either sex. All data regarding milk feeding was downloaded from the automatic calf feeder (*H&L Calfguide*). Whilst in the group hutch, calves had ad-libitum access to starter pellets via a trough. This feed was the same as that offered during their time in the individual hutches. Constant access to fresh water was maintained via a drinking bowl. Male calves were housed in a separate group hutch to the female calves but the stocking density of the group pens was kept at 14 per group regardless of the sex of the calf.

2.2.4 Measurements

Measurements were taken from each calf from the day it entered the group hutch (7.9 days of age ± 0.09) (mean ±se) until day 40 of age when the weaning process commenced. Every day between 0800 and 1000h, all calves within the group hutch were collected and temporarily penned in the concrete apron at the front of the group hutch (5.1m by 1.9m) where they remained until the measurements on all the calves were completed. Each calf was removed from the temporary pen and placed in a modified weigh crate. The modifications to the weigh crate included the removal of the weigh bars and display, and the partial removal of the left panel to allow access to the calf. The modified weigh crate was placed in the alley outside the group hutch. The first procedure carried out on each calf whilst restrained in the modified weigh crate was the assessment of rectal temperature. This measurement was taken using a newly purchased digital thermometer (*Genia Digiflash, St. Hilaire de Chaléons, France*) which was inserted to a depth of 6cm. The head of the calf was held by an operative to allow a thermal image to be taken by the experimenter. Immediately before the capture of the thermal image, air temperature (^{o}C), wind speed (m/s) and relative humidity (%) were noted from a Kestrel 4500 weather meter (*Nielsen-Kellerman, Birmingham, MI, USA*) with air temperature and relative humidity programmed into the image details. The weather meter that was calibrated annually, was positioned at the level of the calf's head.

2.2.5 Thermal images

Thermal images were taken using a FLIR SC620 thermal camera (*FLIR Comp, Boston, MA, USA*) 0.5m from the subject calf at an angle of between 45^{o} and 90^{o} with an emissivity of 0.98 and a reflective temperature of 15^{o}C. The thermal camera had a pixel resolution of 640x480. The thermal image was taken from the area in and around the medial canthus of the left eye of the calf (Figure 2.1), as this area of the body has been shown to be a proxy measure for core temperature in humans (Childs et al., 2012). One thermal image per calf was taken per day on study. Over the duration of the study, three trained operators acquired the thermal images. The coefficient of variation for the repeatability of thermal imaging and between operator variability was calculated by the following equation: CV (%) = (sd/mean) x 100; and found to be 0.41% and 0.56% respectively. In this study, the operator variability was slightly higher than that reported in Byrne et al. (2017) and Scoley et al. (2018). After each imaging session, the thermal images were transferred from the SD memory card of the thermal camera to a computer. Using ThermaCAM™ Researcher Professional 2.10 software (*FLIR Systems, Danderyd, Sweden*), the colour palette was changed from the default Iron to Rainbow900 to allow for clearer observation. An area 2.5cm in diameter was imposed over the medial canthus area of each image to allow the software to extract the maximum temperature (Byrne et al., 2017) within that area.

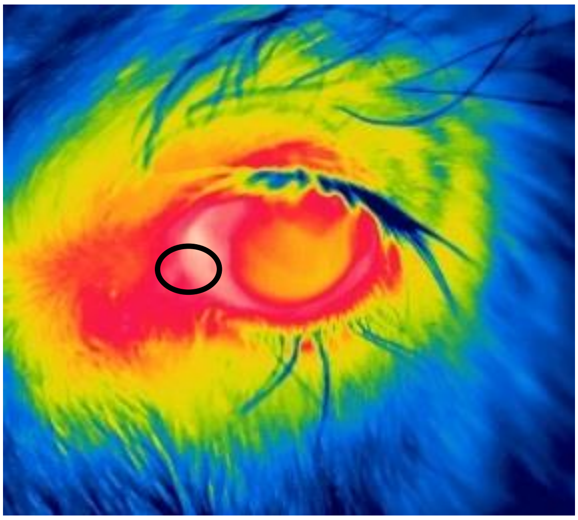


Figure 2.1 Thermal image of inner calf eye, black circle indicating area of interest (in and around medial canthus)

2.2.6 Secondary group examination

During the data analysis, an effect of calf sex on temperature was discovered. However, as female calves were fed on a different regime than male calves, the effect of sex and feeding regime could not be differentiated. Therefore a secondary group of 22 calves were examined further. This non-study group consisted of 10 male and 12 female calves of similar age to those used in this study. Thermal images were taken alongside rectal temperatures using the method explained previously and processed in the same way. The calves examined were all fed on the same milk feeding programme regardless of sex.

2.2.7 Data analysis

All statistical analysis was conducted using R-software (R Core Team, 2016). On any day, if calves had a missing value for rectal temperature or thermal image temperature, these records were excluded from the dataset for analysis. This resulted in a final dataset of 3,737 pairs of rectal and thermal image temperature values from 125 calves. This did not include any data from the 22 calves from the secondary group. All continuous measures were assessed for normal distribution.

2.2.7.1 Water Vapour Density

Water vapour density (g/m^3) was calculated retrospectively from the recorded air temperature and relative humidity using the following calculations (Vaisala, 2013);

$$A = C \cdot P_w/T$$

where A represents the absolute humidity (water vapour density) (g/m^3), C represents a constant of 2.16679 (gK/J), P_w represents vapour pressure (Pa), T represents air temperature (K). P_w was derived from the following calculation;

$$P_w = P_{ws}\ (T) * RH/100$$

where Pw represents vapour pressure (hPa), P_{ws} represents water saturation vapour pressure (hPa), T represents air temperature (^{o}C) and RH represents relative humidity (%). P_{ws} was derived from the following calculation;

$$P_{ws} = A * 10^{(m\ T/T+Tn)}$$

where P_{ws} represents water saturation vapour pressure (hPa), A represents a constant (6.116441), m represents another constant (7.591386), T represents air temperature (^{o}C) and T_n represented the constant 240.7263.

The constant values for A, m and T_n were selected for the temperature range of -20 to +50^{o}C.

2.2.7.2 Energy from milk replacer

The energy provided from the consumption of milk replacer was based on the manufacturer specifications of the milk replacer and the quantity of milk replacer (kg) consumed by the calf between recording sessions. The following calculation was used to calculate the metabolisable energy supplied from 1kg of the milk replacer:

$ME(MH/kg) = \{[0.057 * \%\ crude\ protein] + [0.092 * \%\ fat] + [0.0395 * \%\ lactose]\} * 3.77$ (AHDB, 2020)

The energy provided from the consumption of milk replacer was then calculated for each calf per day by multiplying the ME from the milk replacer by the quantity of milk replacer consumed by each calf between recording sessions.

2.2.7.3 Statistical analysis

Using the '*lme4*' package (Bates et al., 2015), a multivariable linear mixed effects model to predict core body temperature was constructed by applying the stepwise manual backward elimination method. Due to the repeated measurements of each calf, calf ID was included in the model as a random effect along with thermal image temperature, air temperature, wind speed and the energy provided from the consumption of milk replacer. The maximal model included the variables thermal image temperature, air temperature, relative humidity, water vapour density, wind speed, energy provided from the consumption of milk replacer between recording sessions, age of calf and sex of calf. Explanatory variables were then removed in turn, the one with the lowest significance/highest P value first. This was carried out until only variables that were statistically significant (p value <0.05) remained in the model.

2.2.7.4 Predictive equation creation

The dataset was randomly divided into two subsets, with half being used to create the model (training dataset, 1859 observations) and the remaining half being used to validate the model created (testing dataset, 1878 observations). This split (50:50) was seen as being an acceptable method to ensure that some of the observations for rectal temperature ≥39.5^{o}C were present in both datasets. The dataset was divided by calf ID (training dataset: 50 male, 12 female; testing dataset: 50 male, 13 female)

The '*lme4*' package (Bates et al., 2015) was used to create the multivariable linear mixed model with the explanatory variables from the previous model refinement and calf ID as the random effect to cope with the repeated measurement of the same calf. The explanatory variables of air temperature, wind speed and thermal image temperature were centred to allow the model to converge. The training data set was used to generate a predictive model, which was then applied to the testing data set, but excluding the random effects to generate predicted values for rectal temperature. The predicted and actual rectal temperatures were compared.

2.2.7.5 Effectiveness of equation

An analysis of specificity and sensitivity was carried out using '*caret*' package (Kuhn, 2018) to examine how reliable the created equation was at predicting core body temperature over 38.8^{o}C (median temperature from training dataset) and 39.5^{o}C that represented pyrexia in calves (Knauer et al., 2016; Mahendran et al., 2017a).

2.3 Results

2.3.1 Relationship between core body and thermal image temperature

Regardless of the sex of the calf, thermal image temperature was 2.1^{o}C ±0.01 (mean ±se) lower than rectal temperature. The mean rectal temperature of the study calves was 38.8^{o}C ± 0.01 (mean ±se) (Table 2.1). In the final dataset, 394 of the 3737 observations had a rectal temperature that was greater or equal to 39.5^{o}C. There was no variation in rectal temperature by the age of the calf (Table 2.2).

Table 2.1 Description of rectal temperature (^{o}C) and thermal image temperature (^{o}C) by sex of calf

Sex of Calf	Rectal temperature (^{o}C) (mean ± se)	Thermal Image temperature (^{o}C) (mean ± se)
Male	38.9 ±0.01	36.7 ±0.01
Female	38. 7 ±0.02	36.6 ±0.02
All	38.8 ±0.01	36.7 ±0.01

Table 2.2 Description of rectal temperature and thermal image temperature by age of calf

Age (days)	No. observations	Rectal temperature (^{o}C) (mean ± se)	Thermal image temperature (^{o}C) (mean ± se)
<16	953	38.8 ± 0.02	36.6 ± 0.02
17 – 25	1027	38.9 ± 0.02	36.7 ± 0.02
26-33	898	38.9 ± 0.02	36.7 ± 0.02
>33	859	38.8 ± 0.02	36.8 ± 0.02

A comparison of the mean rectal temperature by calf sex showed that the rectal temperature of male calves was on average 0.2^{o}C higher than female calves. When plotted, rectal temperature showed a weak positive relationship (r= 0.28) with the thermal image temperature (Figure 2.2).

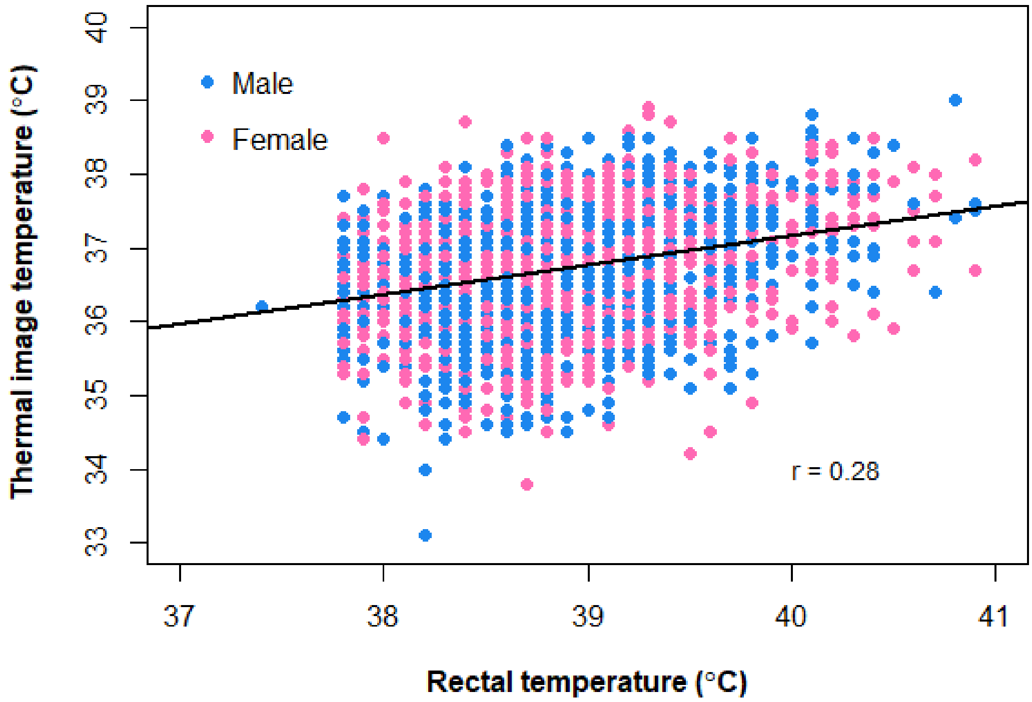


Figure 2.2 Correlation of thermal image temperature (°C) and rectal temperature (°C) of 100 male and 25 female calves

As the sex of the calf and the feeding regime were confounded in the main study group, the data from the secondary group inspection (where both male and female calves were fed on the same regime) was tested to determine whether a real sex difference existed. Analysis of this data showed that the mean rectal temperature of the calves was 38.7°C ±0.07 (mean ±se), with the mean rectal temperature for the male calves of 38.8°C ±0.10 (mean ±se) and the female calves 38.7°C ±0.09 (mean ±se) ($F_{(1, 20)} = 0.366$, *p=0.552*). There was no difference between sexes of calf in terms of thermal image temperature (male calves 37.3 ±0.12°C; female calves 37.3±0.21°C (mean ±se), $F_{(1, 20)} = 0$, *p=0.983*).

2.3.2 Climatic and diet variables

Air temperatures during data collection ranged from -0.8 ^oC to 22.6 ^oC (10.9^oC ±0.07, mean ± se) with wind speed ranging from 0.0m/s to 2.3m/s (0.2m/s ± 0.01, mean ± se). Relative humidity ranged from 49.0% to 100.0% (85.5% ± 0.22, mean ± se) and from the calculation, water vapour density (WVD) ranged from 3.8g/m^3 to 15.7g/m^3 (8.8g/m^3 ± 0.04, mean ± se). Regardless of the sex of the calf, the energy provided from the comsumption of milk replacer ranged from 0.0MJ/d to 42.7MJ/d (16.6MJ/d ±0.11 (mean ±se)). The mean energy provided from the consumption of milk replacer by the male calves was 17.2MJ/d ±0.13 (mean ±se) and 14.4MJ/d ±0.15 (mean ±se) for the female calves.

2.3.3 Predictive model

The parameters of age, relative humidity and water vapour density were dropped from the maximal multivariable model (*p=0.715, p=0.486, p=0.218* respectively) (Table 2.3). As a result of this, only the variables of thermal image temperature, air temperature, wind speed, energy from consumed milk replacer (Milk Energy) and sex of calf were retained at this stage of the analysis. However, sex of calf was later dropped from the model as a result of the secondary group examination.

Table 2.3 Estimates of effect of variables

Fixed Effect	Estimate	S.E. of Estimate	P value
Intercept	26.970	0.569	<0.001
Thermal Image temperature (^oC)	0.336	0.015	<0.001
Air temperature (^oC)	-0.037	0.011	0.001
Relative humidity (%)	-0.002	0.002	0.486
Water vapour density (g/m^3)	0.026	0.021	0.218
Wind speed (m/s)	0.154	0.022	<0.001
Sex	-0.201	0.044	<0.001
Milk Energy (MJ/d)	-0.008	0.001	<0.001
Age (days)	0.000	0.001	0.715

2.3.4 Predictive equation

From the training dataset, the estimates in Table 2.4 were derived; however these values were based on the centred values for the fixed effects. In the first instance, the values were centred to allow convergence. Centring was carried out by subtracting the mean of the variable from each of the datapoints of that variable. To obtain the intercept of 26.467 values were un-centred.

The final predictive equation created was:

*Rectal temperature = 26.467 + 0.347 *thermal image temperature – 0.026* air temperature +0.122* wind speed – 0.007*Milk energy*

There was a moderate relationship (r = 0.32) between the predicted values generated from the testing dataset based on the model from the training dataset and the actual observed rectal temperatures which is an improvement from the original correlation (r = 0.28) (Figure 2.3).

Table 2.4 Estimates of effects of (centred) variables based on training data dataset

Fixed Effects	Estimate	SE of Estimate	P value
Intercept	38.823	0.026	<0.001
CentImageTemp	0.347	0.034	<0.001
CentATemp	-0.026	0.006	<0.001
CentWS	0.122	0.035	<0.001
CentMilkEnergy	-0.007	0.002	0.002

CentImageTemp – (centred) thermal image temperature, CentATemp – (centred) air temperature, CentWS – (centred) wind speed, CentMilkEnergy – (centred) milk energy

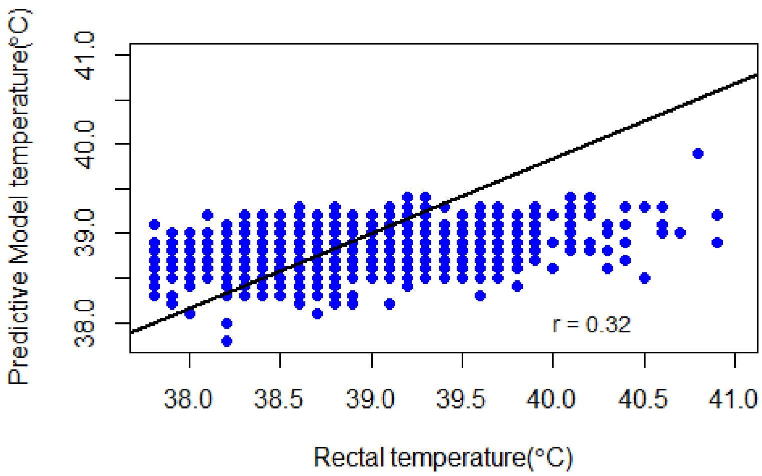


Figure 2.3 Correlation of predicted temperature (^{o}C) from created model and rectal temperature (^{o}C) (data from 63 calves)

2.3.5 Effectiveness of thermal imaging as a predictor of rectal temperature

Sensitivity (Sn) and specificity (Sp) analysis was carried out using the testing dataset to examine how well the model worked at two temperatures, 38.8^{o}C (median temperature of the dataset) and 39.5^{o}C to represent pyrexia (Table 2.5). The model was less sensitive at predicting rectal temperatures >39.5^{o}C than at 38.8^{o}C (Sn = 0.00 (39.5^{o}C), 0.73 (38.8^{o}C)). Therefore, it was poor at detecting calves that did in fact have a rectal temperature greater than 39.5^{o}C (true positive).

Table 2.5 Sensitivity & specificity analysis of testing dataset at median temperature (38.8°C) and pyrexia (39.5°C)

	38.8°C	*39.5°C*
True positive	755	1
False positive	486	0
True negative	351	1664
False negative	286	213
Predictive value positive	0.61	1.00
Predictive value negative	0.55	0.89
Sensitivity	0.73	0.00
Specificity	0.42	1.00

2.4 Discussion

2.4.1 Relationship between core body and thermal image temperature

This study aimed to establish if there was a relationship between rectal temperature and the temperature extracted from a thermal image. It was found that there was a weak correlation between rectal temperature and thermal image temperature of 0.28. A similar correlation (0.24) was found by Scoley et al. (2018). George et al. (2014) suggest a much stronger relationship with a correlation of 0.58. A possible reason for the variation in this relationship could be due to the age of animal used in both studies. Calves between 7 and 40 days of age were used in this study, whereas (George et al., 2014) used multiparous cows. Another possible reason for the difference could be due to thermoregulation in the calf. Hill et al. (2016) demonstrated that the body temperature of calves was at its lowest around 0800h, and at its maximum between 1700 and 2200h. The study by George et al. (2014) took readings slightly later in the day than this study (0900 to 1200h, this study 0800 to 1000h). Also the core body temperature of an adult cow is liable to be more stable than that of a young calf (Piccione, et al., 2003).

It has been suggested that rectal temperature is a true measure of core body temperature but it may vary with thermometer placement (Burfeind et al., 2010; Naylor et al., 2012). However, it is the best measure available and is used by veterinary professionals to detect pyrexia. As far as the authors are aware, there is no known agreed procedure for the accurate use of digital thermometers. Likewise it has been shown that eye temperature varies with environmental influences such as wind speed (Church et al., 2014). However, there are no other easily accessible areas of the body that have such a direct relationship with the core body. Scoley et al. (2018) explored the possibility of taking thermal images of the area around the rectum. However, as they correctly state, this still requires the calf being handled and the tail being raised as well as allowing for any residual heat to dissipate from where the tail had been resting.

A similar issue is raised when selecting an area of the body to estimate core body temperature with thermal imaging. Teunissen and Daanen (2011) question whether or not the inner canthus of the eye is the most suitable area to take a thermal image of to estimate core body temperature as they found that there was often an inconsistent relationship with the thermal image temperature of this area compared to the temperature of the oesophagus in humans. Scoley et al. (2018) used the rectal area of calves as another anatomical region to take thermal images of. In that study, they found a correlation of 0.38 between the thermal image temperature of that area and core body temperature. However, to obtain the thermal image, it required the tail of the calf to be raised which highlights some practical issues about using this area.

Studies by Byrne et al. (2017) and Scoley et al. (2018) both explored the repeatability of thermal imaging. Both of these studies concluded that the standard error of the maximum temperature gained through thermal imaging of the eye can be reduced if more than one image is taken with both studies making the recommendation that three images be taken. These results had not been available or published at the time this study commenced.

In terms of the temperatures gained from the thermal images, it could be said that the temperatures gained were influenced slightly by the stress of handling the calf for the purpose of obtaining an image. However, compared to other studies, such as Scoley et al. (2018; 2019) the thermal image temperatures obtained in this study were much lower. A suggested reason for this could be the time of year and housing conditions. Scoley et al. (2018; 2019) conducted their studies between September and December in calf accomodation that one assumes is within a solid sided building whereas in this study, the thermal images were taken from January to September within an open sided building. There would have been potentially more external influences on the thermal image temperatures gained in this study than the other studies.

2.4.2 Climatic and diet variables and the predictive model

Both air temperature and wind speed were shown to be of statistical significance ($p<0.001$) in the final model to predict rectal temperature using thermal image temperature. These findings corroborate the ideas of Church et al*.* (2014), who suggested that wind speed affected the thermal image temperature. However, according to Gloster et al*.* (2011), air temperature had no significant effect on the temperature gained through thermal imaging of the eye ($p>0.05$), despite showing a difference between rectal temperature and thermal image temperature of the eye of around 2^{o}C which is similar to the present study.

From this study, it can be seen that there is a moderate relationship between rectal temperature and predictive rectal temperature using the model created. However, it can also be seen in Figure 2.3 that there is large variation surrounding the predicted values that was not captured by the measurements taken in this study. One possible source of variation that was not assessed was the effect of solar radiation on the surface temperature of the eye of the calves. Studies by Paterson et al*.* (2011) found that levels of solar radiation was statistically significant in their study and included in their model.

Other possible reasons for this variation could be the distance from which the thermal image was taken, the angle at which the thermal image was taken and surrounding environment. Okada et al. (2013) have shown that the distance between the object of interest and the imaging device is of importance to obtain an accurate thermal image temperature. The distance chosen for this study (0.5m) may possibly not have been adequate. Okada et al. (2013) noted that the greater the distance, the lower the detected temperature by thermal imaging, especially over 2.0m. This could also account for the lack of agreement in the correlation between thermal image temperature and rectal temperature between this study and George et al. (2014). This study used a distance of 0.5m whereas George et al. (2014) was slightly closer at 0.3m. Jiao et al. (2016) have also shown the angle at which the thermal image is taken is also of importance. It was not possible to fully restrain the calf's head during measurements, and therefore the angle at which the thermal image was taken would vary slightly depending on calf movement. This may have resulted in some variability in the reading obtained.

Carroll et al. (2012) mention that environmental and physical stressors (e.g. air temperature, wind speed, handling) can induce metabolic changes in the calf which in turn can affect temperature regulation. Therefore, it is justified to include diet in the model as this would take into consideration that the calf is metabolically immature in terms of temperature regulation. In future work consideration should be given to recording concentrate and water intakes as well as milk intakes as they will be linked to rumen development and function which may have a possible temperature effect.

2.4.3 Reliability of model

The sensitivity and specificity analysis suggest that the model could not correctly identify incidences of pyrexia (i.e. core body temperatures over 39.5^{o}C; Sn =0.00). At this temperature threshold, the model only identified 1 incidence of pyrexia correctly out of the 214. Naturally, the sensitivity of the model improves at a lower temperature threshold (e.g. 38.8^{o}C) due to more incidences being included in the sample. At the temperature threshold level of 38.8^{o}C, the model correctly identified 755 incidences above this temperature out of 1041. However, at the 39.5^{o}C threshold, the predictive equation could correctly exclude the condition (i.e. identify a temperature below 39.5^{o}C) when it was not present (Sp=1.00). This can also been seen by the negative predictive value (NPV) (0.89) meaning that 89% of cases truly had a core body temperature below 39.5^{o}C. The NPV will increase and the PPV will decrease as the prevalence of the condition decreases. Using the 39.5^{o}C threshold, the mean daily prevalence of pyrexia in the calves used in this study was 10.2% ±0.56 (mean ±se) and whilst there are no UK figures available on the prevalence of pyrexia in calves, the authors' consider that the sample population was typical of UK dairy calf populations. A higher prevalence of pyrexia in the calves might have strengthened the model to predict a body temperature >39.5^{o}C. Therefore, this would suggest that with the incorporation of the identified parameters, thermal imaging could potentially be used to identify a calf with a core body temperature that is not deemed pyretic.

Despite the apparent positive aspects of the use of thermal imaging such as the non-invasive nature of the technique, it does not appear to be at a stage of functionality where it can be used to detect pyretic calves reliably. Further work should investigate other potential sources of variation that could help improve the correlation between core body temperature via rectal temperature and thermal image temperature. Another area that should also be considered is the use of repeated image measurements via automation where the thermal images can be captured at an area that the calf would regularly visit such as a milk feed station. Through this means of data capture, there would be the possibility of the health status of the calf being determined through the use of machine learning.

Perhaps the data captured from the thermal images of this study represent a completely separate phenotype, such as the prediction of ill-health or stress, and not that which reflects core body temperature.

2.5 Conclusion

Based on the results from this study, using a model that includes thermal image temperature and corrected for the environmental parameters of air temperature and wind speed as well as diet, there was a slight improvement in its correlation with rectal temperature from the original correlation of thermal image temperature and rectal temperature. Further investigation under farm conditions would be needed to explore the possibility of explaining the relationship. However, from the sensitivity and specificity analysis, there is the possibility that thermal imaging could be used as a "proxy measure" of body temperature to identify non-pyretic animals, but it would be more important to identify the pyrexic animal with high accuracy and repeatability.

Chapter 3: The behavioural response of young pre-weaned dairy calves to wind speed and air temperature

3.1 Introduction

Dairy calves are born into an environment with a temperature that is a lot lower than that of the temperature *in utero* (Vermorel, et al., 1983; Rowan, 1992; Carstens, 1994). Although they are born with functional thermoregulatory mechanisms, their ability to regulate body temperature is immature. This means that they are at an increased risk of death or reduced growth as a result of exposure to adverse weather conditions or fluctuations in air temperature (Martin et al., 1975). One of the main objectives for housing calves is to assist in alleviating the issue of environmental variation. By housing calves, they can be protected from extremes in climatic and thermal conditions, for example strong wind and low temperatures (Stull and Reynolds, 2008). There are various housing styles and systems available for housing calves such as individual pens within an existing building, an individual hutch that can be placed outside, indoor group pens and group hutches. No matter which type of housing is chosen, the aim should be to provide the young animal with an environment where it does not experience conditions that are too hot or too cold. In technical terms this can be described at the thermal comfort zone (TCZ). The thermal comfort zone is the environmental temperature at which the animal (in this case the calf) is not motivated to perform any thermoregulatory behaviour. Thermal comfort lies within the thermo-neutral zone (Kingma et al., 2014). The thermo-neutral zone is the range of ambient temperature where the metabolic rate of the animal is low and temperature regulation can be accomplished through non-evaporative physical process (Bligh and Johnson, 1973). In essence, there is a balance between heat production and heat loss. In the literature, there is variation in the suggested thermo-neutral zone for a new born calf from 15-25^{o}C (Stull and Reynolds, 2008) to 10-30^{o}C (Webster, 1984). The lower limit of the thermo-neutral zone is referred to as the Lower Critical Temperature (LCT). This is the temperature below which, in this case, the calf needs to increase heat production from its metabolism in order to maintain thermal balance.

The LCT can be affected by wind speed and moisture levels as well as age of calf and plane of nutrition (Webster, 1984). Therefore keeping a calf in a situation with wet bedding material and exposure to draughts can create an environment for the calf where its LCT is much higher than anticipated. Roe (1982) indicates that a draught would be considered to be wind speed above 0.25m/s. In calf housing, sources of draughts are usually from the air inlets. Draughts create a chilling effect on the calf and therefore it needs to increase its heat production. Also, draughts could potentially increase the calf's susceptibility to disease (Webster, 1981) by causing stress on the calf which can inhibit the immune system. However, as Nordlund (2008) states, any type of calf housing should still allow a small volume of fresh air to the area around the calf. This small area surrounding the calf tends to be referred to as the calf's microclimate.

At this stage, it is worth highlighting that a lot of the studies mentioned regarding thermal comfort and LCT are around 40 years old. It would also be fair to comment that the modern day dairy calf will be genetically different from the calves used in such studies through more targeted breeding e.g. breeding for specific linear or production traits but also through different management and feeding programs. Therefore, should the same assumptions about thermal comfort and LCT be made based on 40 year old information? However, findings concerning age and body surface area will still apply today.

The age of the calf is also important in terms of thermoregulation as young calves are sensitive to the cold due to their high body surface to mass ratio and can cause them to lose heat to their environment quickly (Van laer et al., 2014). Body mass increases as the calf gets older and grow (Berman, 2003). This is associated with changes in proportion of body parts, which would lower the body surface to mass ratio.

The behaviour an animal expresses in conditions that are outside the TNZ signifies a response to its environment and occurs in order to minimise or aid heat loss (Rowan, 1992). Baldwin (1973) reports some of the behavioural thermoregulatory responses at high and low ambient temperatures and although not done by the author, these responses could potentially be categorised into four classes. The four classes of response are (i) changes in intakes, (ii) changes in posture and activity, (iii) social and (iv) use of resources (such as bedding material, shade and shelter). At high ambient temperatures, feed intake is reduced and water intake is increased (Roland et al., 2016) whereas at low ambient temperatures there is a need to increase feed intake (Rowan, 1992). In terms of postural and activity related behavioural thermoregulatory responses, at high ambient temperatures, locomotor activity is decreased and the animal can be seen lying down in extended positions. At low ambient temperatures, the animal can be seen slightly hunched. Graunke et al. (2011) found that when it rained, the cattle (which were aged between 3 and 10 years in the study) were observed lying down more than when it was not raining. A similar trend was found by Tripon et al. (2014) whereby calves spent more time lying than standing in the winter than the summer months. Overall, lying could be considered a response to cold environments (Vermorel et al., 1983) due to less body surface area being exposed to the low temperatures, and therefore a mechanism to conserve energy.

Calves seen huddling together could be regarded as a social-related response to low ambient temperatures, whereas calves lying away from one another avoiding body contact would be how they would respond in high ambient temperatures. Although not a social activity, Tripon et al. (2014) found that as the temperature of the air decreased self-grooming increased. During the winter months the calves groomed themselves about six times more compared to in the summer months. This is an example of a behaviour indicating ‘annoyance’.

The avoidance of direct sunlight by the use of shading, nest building behaviour with the available bedding material and looking for shelter by seeking microclimates with high or low ambient temperatures are examples of using resources to display a behavioural thermoregulatory response

(Baldwin, 1973). Webster (1984) highlights that supplying enough dry bedding material for the calf will allow it to nest down in such material and attempt to avoid draughts. A study by Graunke et al. (2011) demonstrated that the cattle used found microclimates that had lower wind speed and higher temperatures rather than being in the open, which matched the findings of Houseal and Olson (1995) and Redbo et al. (2001) who also found that cattle adapted their choice of location to the climatic conditions. Overall it shows that there are some behavioural indicators that could potentially be used to determine whether or not younger cattle (i.e. calves) feel cold.

The aim of this experiment was to examine the behavioural responses of the pre-weaned dairy calf to different wind speeds and air temperatures. The air temperatures used were determined from the literature as those that were considered to be around the TCZ (15^{o}C), around LCT (10^{o}C) and below LCT (5^{o}C). The wind speeds chosen were 0m/s (no wind speed, 'still' conditions), 1.0m/s (low wind speed) and 3.3m/s (high wind speed) to represent conditions that could potentially be experienced within calf housing as well as being above what is considered a draught.

It was hypothesised that the calf would respond more at the higher wind speeds and lower air temperature. In terms of responsiveness, the calf would seek shelter quicker and display more 'annoyance' behaviours under these conditions than at no wind speed and the higher air temperatures.

3.2 Materials and methods

3.2.1 Pilot study

For this experiment, a pilot study was initially conducted before the main study. Due to the availability of animals at that time, only three calves were used for the pilot study. An arena was constructed that could be adjusted in size and this was used to determine the size of the testing arena to use in the main study. The three air temperatures and wind speeds used for the main study were used in the pilot study allowing for the behavioural reactions of interest to be established. At this stage, a testing session of thirty minutes was used. However, it was felt to be too long as the calves had made their choice of location and shown other behavioural responses before the end of the thirty minutes. Therefore the duration of the test session was reduced to twenty minutes.

3.2.2 Main study

3.2.2.1 Animals

The study utilised eighteen pre-weaned Holstein female calves that were temporarily recruited from the dairy herds at SRUC Crichton Royal Farm, Dairy Research & Innovation Centre, Dumfries. The study period for this experiment (Home Office Project Licence PPL P204B097E, SRUC Animal Experimental & Ethics Approval ED AE 05 2017) was between 3 April to 18 May 2017, 8 October to 22 November 2017 and 3 April to 26 May 2018. All calves were reared in accordance to the current farm practice at SRUC Dairy Research & Innovation Centre. All calves were tested in the age range of 7-25 days, as according to Webster, (1974) by around one month of age the LCT of the calf is $0^{o}C$ and therefore is less likely to experience chilling at the range of air temperatures under test.

3.2.2.2 Home pen and feeding

The ‘home pen’ for the calves used in this study consisted of a group Igloo housing system *(Holm & Laue, Westerfeld, Germany)*. The Igloo system consisted of a hand laminated fibre-glass constructed dome (igloo) (3.9m, 4.4m, 2.2m: length, width, height respectively, volume $20m^3$) which provided the calves with shelter, and a roofed pen (5.1m x 5.1m – length & width) which was in front of the igloo. Each pen contained 14 calves of mixed sex and breed. Each pen was equipped with an automatic milk feeder (H&L 100)

(*Holm & Laue, Westerfield, Germany*). When in the group pen, each calf had the opportunity to consume seven litres of reconstituted milk replacer per day at a 15% concentration level. The milk replacer (Omega Gold, *ForFarmers, Suffolk, England)* consisted of 23% crude protein, 18% crude fat, 0.1% crude fibre and 8.5% crude ash. All calves had daily ad-libitum access to starter pellets via a trough, fresh water via a drinking bowl and straw via racks.

3.2.2.3 Test conditions

3.2.2.3.1 Creating desired wind speed

To create the desired wind speed, two 16' diameter pedestal fans (*Homebase, Milton Keynes, England*) were used. If the calf was to be tested at no wind speed (0m/s) then the fans were placed 1.2m from the front of the test pen and not turned on. This distance from the front of the pen was also used for testing calves at low wind speed (1.0m/s) but with the fans turned on. When testing calves at the high wind speed (3.3m/s), the operating fans were placed directly in front of the board at the front of the test pen.

3.2.2.3.2 Determining air temperature

The air temperature conditions selected to undertake the test were naturally occurring, thus testing took place during the time period of the year when temperatures were expected to be variable (i.e. spring and autumn) to allow the range of air temperatures to occur within the test phase for each calf. The day and time of day for testing a calf was selected based on when the desired air temperature (5, 10, 15^{o}C) was anticipated to occur by frequently observing the weather observations for Dumfries, Crichton Royal No2 from the Meteorological Office website (*www.metoffice.gov.uk*).

3.2.2.4 Test arena

The test arena (Figure 3.1 & Figure 3.2) was created in part of the existing calf rearing shed (Figure 3.3), so that the calves tested were familiar with the sounds and smells of this environment. The test arena consisted of two defined sections: test pen and shelter pen, both of equal sizes which were adjacent to each other and could be accessed from the other. It was created from metal hurdles of two sizes (1.25m in length, 1.0m in height and 1.8m in length, 1.0m in height) to form an area 2.5m by 2.5m (length by width). Therefore each of the 2 parts of the test arena (test pen and shelter pen) was 1.25m by 2.5m in dimension.

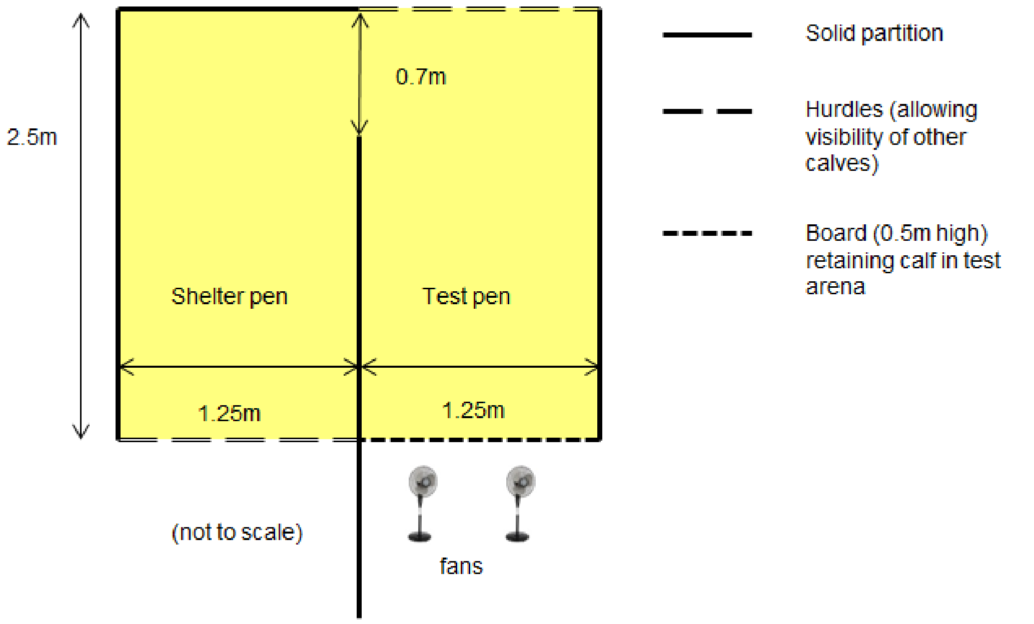


Figure 3.1 Diagram of testing arena

Figure 3.2 Layout of testing arena showing the test pen (on the left) and shelter pen (on the right), position of fans and proximity to other calves

Neither part was roofed to minimise air re-circulation. The perimeters of each pen were fully boarded to create a solid side, with test pen on the right hand side, shelter area on the left hand side. The rear of the shelter area was fully boarded as well. No other parts of either pen had a solid side. Separating both pens was a gate 1.8m in length, again fully boarded to create a solid partition. The positioning of this gate created a space of 0.7m at the rear of both pens, and this allowed the calf to access the shelter pen from the test pen and vice versa. The aim was to achieve the perception of a 'wind tunnel'. To keep the calf within the test arena, a small wooden board (0.5m in height, 1.25m in width) was placed at the lower front of the test pen. It did not obstruct the air flow created from the fans.

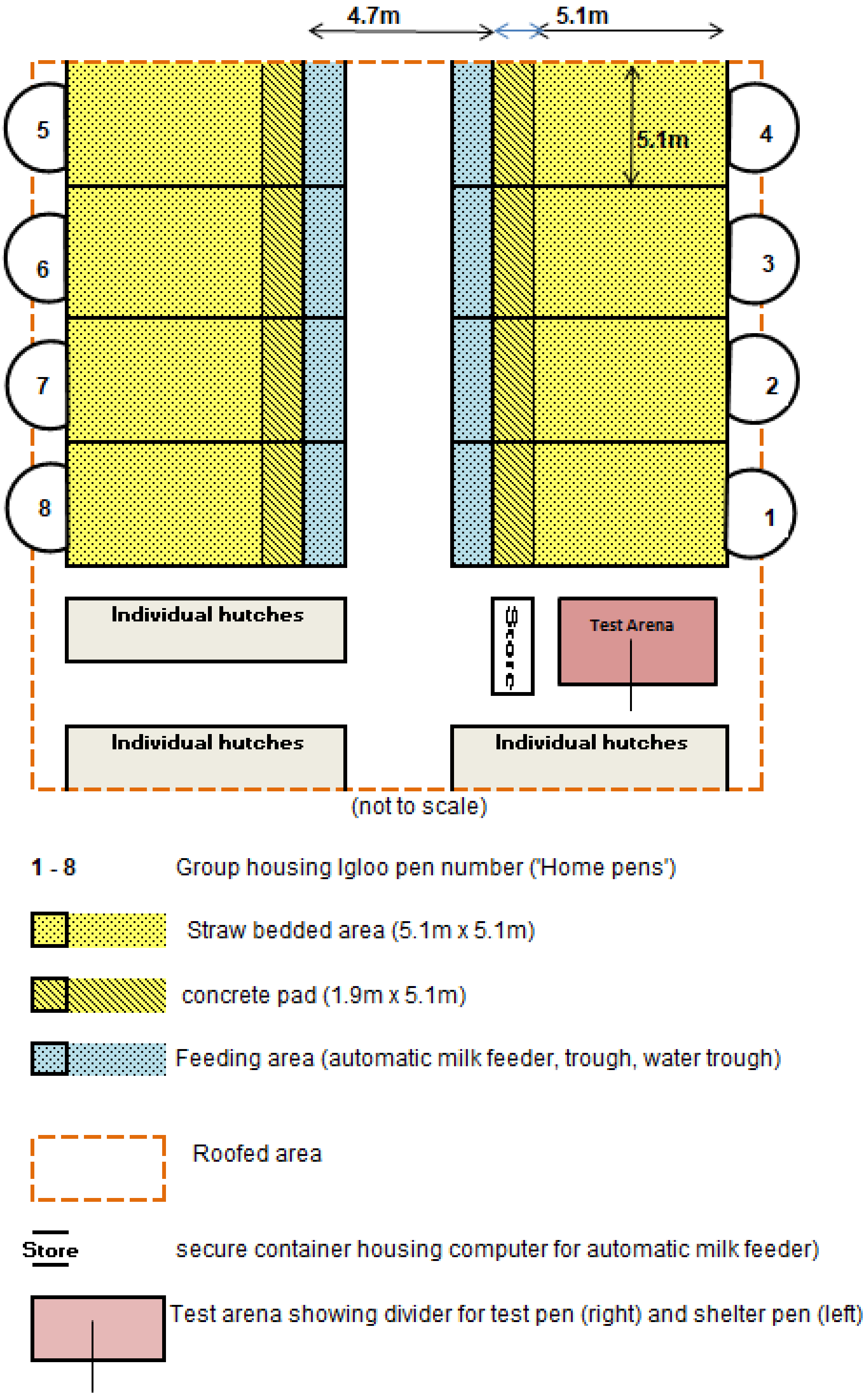


Figure 3.3 Placement of test arena in relation to home pens within existing calf rearing shed at Crichton Royal farm

Both parts of the test arena were bedded with approximately the same amount of straw at each test session and training session. The calf had sight of other calves in the shed at all times during each test session. The experimenter was present in the surrounding area of the test area (but out of view of the test calf) for the duration of the time that the calf was tested so that if the calf displayed any signs of distress (e.g. shivering for more than 2mins) then the test could be immediately terminated.

3.2.2.5 Training

All calves were given two training sessions within the test arena, at least three hours apart and at least 24 hours prior to their initial test session, to familiarise them with the test pen, and make them aware of the shelter pen that allowed them to move away from the wind if they so choose. The training session consisted of the calf being placed in the test pen. The calf remained there for 5mins, which acted as a habituation period. The calf was then allowed to remain in the test pen for a further 5mins. During this time in the test pen, the calf was subjected to a 30s episode of wind of either low or high speed to habituate the calf to the noise created by the fans to be used in the test session. After this 5 minute block, the calf was then moved into the adjacent shelter pen for a further 5mins. The calf was then moved into the test pen again for an additional 5mins, and this time given another 30s episode of wind created from the fans of the alternate speed to which it had initially been subjected to. The overall length of each training session was 25mins. The calves did not have access to food or water throughout the training session but they did have access to the straw bedding material which they could have potentially eaten. During the training sessions the calf could voluntarily move between the test pen and shelter pen. All calves used for the main study were observed voluntarily moving between the test pen and shelter pen. During the training sessions, there was always the possibility that the calf trained itself to react based on its previous exposure.

3.2.2.6 Test process and measurements

Prior to the calf entering the test arena, a weather meter (*Kestrel 4000 weather meter, Nielsen-Kellerman, Birmingham, MI, USA*) was placed 1.10m from the front of the test pen and at a height of 0.7m above the bedding material for the purpose of automatically recording the air temperature and wind speed in the test pen at one minute intervals.

The test calf was removed from its home pen and placed in a calf weigh crate with Tru-Test digital load cells and EziWeigh5 weigh head attached (*Ritchie Agriculture, Forfar, Angus, Scotland*). The liveweight of the calf was noted and subsequently used to calculate body surface area. Next the rectal temperature of the calf was recorded using a new digital thermometer (*Genia Digiflash, Genia, St. Hilaire de Chaléons, France*) and visually assessed using the Wisconsin Calf Health Scoring System (McGuirk, 2008). Any calf with a rectal temperature of 39.5^{o}C and above was not used in the experiment as were calves with an overall Wisconsin health score of 5 and above where the rectal temperature was less than 39.5^{o}C. The mean Wisconsin health score for the study was 1.7 ± 0.09 (mean ± se).

From the weigh crate, the calf was moved to the test arena and placed in the test pen. It was given five minutes to re-familiarise itself with the test arena which allowed the experimenter time to set up the fans and the camcorder (*Canon Legria HF G25, Canon Inc, Tokyo, Japan*) which was used to record every test session. Next, the camcorder was set to record and the calf was positioned in the test pen, standing and facing the direction of the fan. At this point the experimenter left via the front of the test pen and the fans were turned on if that particular test session required it. The test session was then classified as having begun.

After 20mins had elapsed, recording of the test session was stopped. The fans were also switched off, and the test calf was taken back and placed in the weigh crate. The rectal temperature of the calf was re-checked at the end of the test session. An intervention procedure was in place should there have been a deviation of 1^{o}C in core body temperature. No calves used in the study experienced this, so the intervention procedure was not required (rectal temperature pre-test session, 38.7^{o}C ± 0.03; rectal temperature post-test session, 38.8^{o}C ± 0.03 (mean ± se).

From the weigh crate, the test calf was then returned to the home pen. Each calf was tested only once in a 24 hour period.

The nine possible temperature/wind speed combinations were balanced across calves as far as possible, and the order of testing the wind speed was randomised with respect to air temperature. As the air temperatures used in this study were naturally occurring and not artificially created, as well as having a strict cut-off point for the age of the calf, not all calves were able to be tested under every air temperature/wind speed combination in accordance to a Latin square design.

3.2.2.7 Milk feeding data

Milk feeding data was collected from the automatic milk feeders for each calf after every test session. This data comprised of volume of reconstituted milk consumed in 24 hours prior to test session commencing, and the amount of milk replacer consumed in the same 24 hour period. The visit to feed station data was used to calculate the number of minutes from last milk feed until test session.

3.2.2.8 Video recordings

All recorded video test footage was observed and data extracted using The Observer XT12 software (*Noldus Information Technology, Netherlands*). Calf behaviour was noted by following the ethogram detailed in Table 3.1. These reactive behaviours were included as they represented signs of annoyance, irritation and discomfort for the calf in response to acutely stressful conditions (Grøndahl-Nielsen et al., 1999; Stafford and Mellor, 2005; Stilwell et al., 2008). From this observational data, the proportion of time spent in each of the two sections (test pen, shelter pen) was calculated.

Latency of first behavioural reaction (reaction being first expression of the selected behaviours) and latency of first movement between pens (first time the calf physically removes itself from the test pen and moves into the shelter pen) were noted from the test footage. Each calf varied in the behavioural reactions it showed, so the frequencies of each reaction were added together to give an overall response by area of the testing arena.

The number of behavioural reactions per unit of time in the test pen was subsequently calculated by dividing the total number of behavioural reactions displayed only in the test pen by the duration of time that the calf was observed in the test pen throughout each test event.

Table 3.1 Definitions of calf place, posture and reactive behaviours expressed

Description	Definition
Place	
Test pen	Calf defined as being in "Test pen" when the full body of the calf is in the area with wind flow
Shelter pen	Calf defined as being in "Shelter pen" when the full body of the calf is in the area with no wind flow.
Posture	
Standing	Calf defined as standing when a minimum of three feet are in contact with the bedding material
Lying	Calf defined as lying when maximum of two feet are in contact with the bedding material
Reactive behaviours	
Ear Flicking	Calf rapidly moves one or both ears to the front and back independent of a head shake. Each time there is movement in the ears this constitutes an ear flick.
Head Shake	Calf rapidly shakes head from one side to the other. Recorded as a new movement after the head moves slowly or in a resting position.
Tail Flicking	Calf rapidly moves tail from side to side, may include two or three movements. Recorded as a new movement after the tail moves slowly or is in a resting position.
Head/Ear Rubbing	Calf lifts hind leg to scratch top of head or ear with foot.
Self-Grooming	Calf turns muzzle/head/neck towards any other body part followed by up and down or back and forth movements of the muzzle/head/neck.
Whole Body Shake	Calf rapidly shakes entire body. Any repeat of this movement is recorded as a new movement after entire body is in a resting position.

3.2.2.9 Calculations used

Body surface area was calculated using the liveweight recorded just before each test session by the following formula:

$$A = 0.09W^{0.67} \text{ (Webster, 1984)}$$

Where *A* represents body surface area (m^2) and *W* represents body liveweight (Kg).

This calculation was chosen as Berman (2003) states that the estimate of body surface area between different equations is very similar when liveweight is below 100kg.

3.2.2.10 Statistical analysis

All statistical analyses were conducted using R-software program (R Core Team, 2016). All the data were checked for normality. The dependent variables were the proportion of time in the test pen (PropTP), latency of first movement between the test pen into the shelter pen (Lat1Place), total behavioural reactions expressed in the test pen (TBehTP), latency of first behavioural reaction (Lat1React) and the number of behavioural reactions in the test pen by duration of time in the test pen (RBTP).

The independent variable air temperature had three levels (5^oC, 10^oC, 15^oC), as had wind speed (0m/s, 1m/s, 3.3m/s). The other independent variables were categorised by using above and below median values; relative humidity (factor, 2 levels; ≤ 85.6%, >85.6%), body surface area (factor, 2 levels; ≤1.15m^2, >1.15m^2), minutes between last milk feed and test session (factor, 2 levels; ≤112mins, >112mins), volume of reconstituted milk consumed in past 24 hours (factor, 2 levels; ≤5.8l, >5.8l), quantity of calf milk replacer (CMR) consumed in past 24 hours (factor, 2 levels; ≤0.96kg, >0.96kg) and the age of calf at the test session (factor, 2 levels; ≤14days, >14days).

Using the 'l*me4*' package (Bates et al., 2015), all the independent variables were screened using univariable models for association with the dependent variables with calf as a random effect using *glmer* function. Coefficients that had a P value of less than 0.2 were carried forward to a multivariable linear mixed model. The multivariable linear mixed model was carried out using backward step selection. All the dependent variables were modelled using a Poisson distribution.

A subset of the data was created to only include data from 0m/s (i.e. no wind treatment, absence of wind) to look at the effect of air temperature under still conditions. The method described previously was also applied to this dataset.

Post hoc Tukey tests were used to obtain significance between factor levels of significant variables only.

3.3 Results

3.3.1 Effect of wind speed

The calves were on average, of similar age when tested at the three different wind speed conditions (Table 3.2).

Table 3.2 Description of age (days) at which calf was tested - wind speed (m/s)

Wind speed (m/s)	No. of test events	Mean age ± sd (d)	Min Age (d)	Max Age (d)
0	45	15.1 ±5.74	7	25
1.0	42	15.2 ±4.91	8	25
3.3	44	14.4 ±4.70	7	25

As the wind speed increased, calves spent a smaller proportion of their time in the test pen (68% (0.68) ±0.047 for 0m/s, 57% (0.57) ± 0.049 for 1.0 m/s and 44% (0.44) ±0.048 for 3.3 m/s (mean ±se)) (Figure 3.4). There was no significant effect of the highest wind speed on PropTP (*p=0.120*).

As the wind speed increased, calves took less time to move between the test pen and shelter pen for the first time (540.2s ±70.88 for 0m/s, 462.4s ± 64.97

for 1.0 m/s and 237.0s ±53.18 for 3.3 m/s (mean ±se)) (Figure 3.5). There was a significant effect of wind speed on Lat1Place (*p=0.011*).

Following post-hoc tests, there was a significant difference found between 0 and 3.3m/s (*p=0.011*).

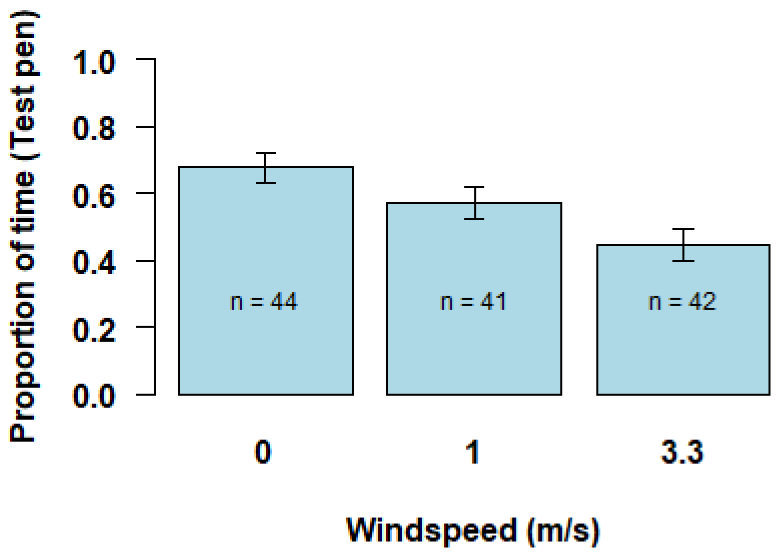


Figure 3.4 Proportion of time in test pen (n= number of test sessions per wind speed)

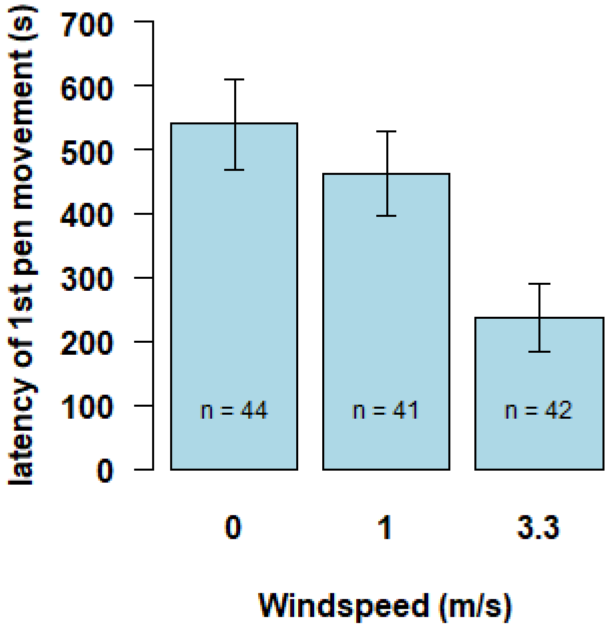


Figure 3.5 Effect of wind speed (m/s) on latency of 1st movement between pens (test and shelter) (n= number of test sessions per wind speed)

As wind speed increased, the total number of behavioural reactions from the calves in the test pen increased (0m/s: 6.5 behavioural reactions ± 0.96, 1m/s: 14.4 behavioural reactions ± 2.46, 3.3m/s: 19.8 behavioural reactions ± 2.40 (mean ± se)) (Figure 3.6). There was a significant effect of wind speed on TbehTP ($p<0.001$) with a significant difference found between 0 and 1m/s ($p=0.024$) and 0 and 3.3m/s ($p<0.001$).

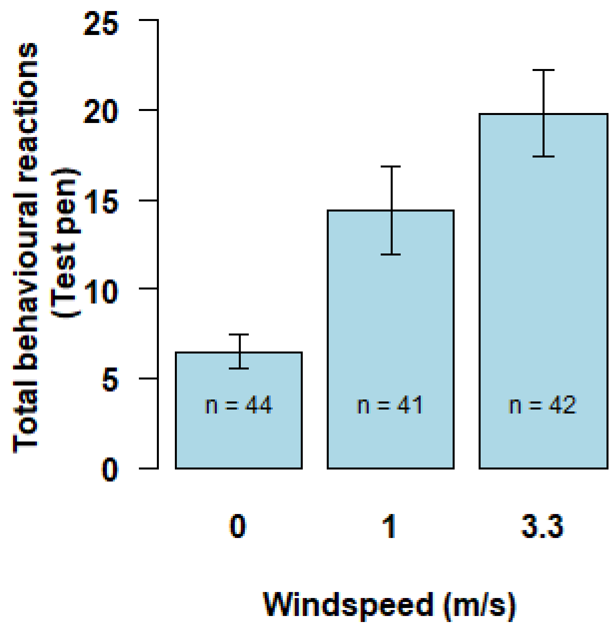


Figure 3.6 Total number of behavioural reactions in the test pen by wind speed (n= number of test sessions per wind speed)

Calves took less time to display any of the behavioural reactions as wind speed increased (205.7s ±49.91 for 0m/s, 90.9s ± 40.68 for 1.0 m/s and 2.7s ±0.13 for 3.3 m/s (mean ±se)) (Figure 3.7). There was a significant effect of high wind speed on Lat1React ($p<0.001$). There was a significant difference found between 0 and 1m/s ($p=0.045$), 0 and 3.3m/s ($p<0.001$) and between 1 and 3.3m/s ($p<0.001$).

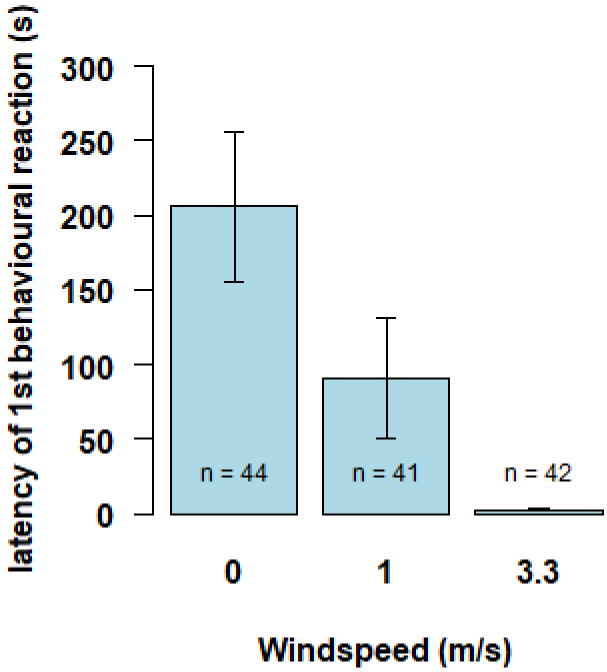


Figure 3.7 Latency of 1st behavioural reaction by wind speed (n= number of test session per wind speed)

Calves displayed more behavioural reactions per unit of time in the test pen as wind speed increased (0.008 behavioural reactions/s ±0.0011 for 0m/s, 0.021 behavioural reactions/s ± 0.0028 for 1.0 m/s and 0.063 behavioural reactions/s ±0.0084 for 3.3 m/s (mean ±se)). There was a significant effect of high wind speed on RBTP ($p<0.001$). There was a significant difference found between 0 and 1m/s ($p=0.002$), 0 and 3.3m/s ($p<0.001$) and between 1 and 3.3m/s ($p=0.002$).

There was no significant interaction between wind speed and temperature for any of the dependent variables and between wind speed and the other significant independent variables (Table 3.3).

Table 3.3 Interaction of wind speed and temperature and wind speed and body surface area

Dependent variable	*Chisq*	*df*	*P value*
Interaction- Wind speed : Temperature			
PropTP	1.399	4	0.844
Lat1Place	2.071	4	0.723
TbehTP	0.914	4	0.923
Lat1React	1.177	4	0.881
RBTP	0.310	4	0.989
Interaction – Wind speed : Body Surace Area			
PropTP	1.089	2	0.580
TBehTP	0.020	2	0.990

3.3.2 Effect of air temperature

As previously mentioned, the air temperatures were all naturally occurring. Table 3.4 illustrates how close to the three temperature levels the test events were conducted under.

Table 3.4 Mean recorded temperatures under which test events were conducted

Temperature (^{o}C)	Recorded Temperature (^{o}C) Mean ± sd
5	5.8 ±0.88
10	10.5 ±0.84
15	16.1 ±1.41

Table 3.5 illustrates that when tested at 5^{o}C, the calves were slightly younger than when they were tested at 10^{o}C and 15^{o}C.

Table 3.5 Description of age (days) at which calf was tested – air temperature (^{o}C)

Temperature (^{o}C)	No. of test events	Mean age ± sd (d)	Min age (d)	Max age (d)
5	42	13.3 ±4.14	7	23
10	51	15.3 ±2.27	7	25
15	38	16.1 ±5.57	7	25

There was no significant effect of air temperature on any of the dependent variables when wind speed was included in the model (Table 3.6).

Table 3.6 Effect of air temperature on dependent variables when wind speed is included in the final model

Dependent variable	*Chisq*	*df*	*P value*
PropTP	0.433	2	0.805
Lat1Place	1.688	2	0.430
TbehTP	0.588	2	0.745
Lat1React	0.147	2	0.923
RBTP	0.017	2	0.992

In the absence of wind (i.e. data collected at 0.0 m/s, 'still' conditions), there was also no significant effect of air temperatures on PropTP, Lat1Place, TbehTP, Lat1React or RBTP (Table 3.7). The results show that there is little evidence to suggest that temperature alone was affecting the behaviour of the calf.

Table 3.7 Effect of air temperature in the absence of wind on dependent variables

Dependent variable	*Chisq*	*df*	*P value*
PropTP	0.315	2	0.854
Lat1Place	1.792	2	0.408
TbehTP	2.667	2	0.264
Lat1React	3.663	2	0.160
RBTP	0.101	2	0.951

3.3.3 Effect of other independent variables

The test calves drank 5.3 l ± 1.62 (mean±sd) of reconstituted milk in the 24h prior to the test session and consumed 0.9kg ± 0.29 (mean±sd) of milk replacer. The test calves had also not consumed milk for 198.6 min ±208.58 (mean ±sd) before the test session.

There was no significant effect of the volume of reconstituted milk consumed in 24 hours prior to the test session commencing, the amount of milk replacer consumed in the same time period, the number of minutes from last milk feed until test session, age of the calf or relative humidity on PropTP, Lat1Place, TbehTP, Lat1React or RBTP when wind speed and temperature were included in the model (Table 3.8) or in the absence of wind (Table 3.9).

Table 3.8 Effect of independent variables on the dependent variables with wind

Variable	*Chisq*	*df*	*P value*
PropTP:			
24hVol	0.239	1	0.625
24hKgCMR	0.672	1	0.412
Minslastfed	0.577	1	0.448
Age	1.033	1	0.310
RH	0.081	1	0.776
Lat1Place:			
24hVol	0.000	1	0.979
24hKgCMR	0.020	1	0.888
Minslastfed	1.324	1	0.250
Age	1.292	1	0.256
RH	0.348	1	0.555
TBehTP:			
24hVol	0.114	1	0.736
24hKgCMR	0.000	1	0.989
Minslastfed	0.710	1	0.399
Age	0.826	1	0.363
RH	0.013	1	0.909
Lat1React:			
24hVol	0.574	1	0.449
24hKgCMR	1.456	1	0.228
Minslastfed	0.095	1	0.758
Age	0.284	1	0.594
RH	0.663	1	0.415
RBTP:			
24hVol	0.009	1	0.927
24hKgCMR	0.031	1	0.859
Minslastfed	0.643	1	0.423
Age	0.310	1	0.578
RH	0.005	1	0.946

Table 3.9 Effect of independent variables on the dependent variables in the absence of wind

Variable	*Chisq*	*df*	*P value*
PropTP:			
24hVol	0.061	1	0.806
24hKgCMR	0.061	1	0.805
Minslastfed	0.000	1	1.000
Age	0.061	1	0.806
RH	0.242	1	0.623
Lat1Place:			
24hVol	0.242	1	0.623
24hKgCMR	0.244	1	0.621
Minslastfed	0.061	1	0.806
Age	1.503	1	0.220
RH	0.544	1	0.461
TBehTP:			
24hVol	0.603	1	0.438
24hKgCMR	0.126	1	0.723
Minslastfed	0.000	1	1.000
Age	1.353	1	0.245
RH	0.038	1	0.846
Lat1React:			
24hVol	0.000	1	1.000
24hKgCMR	0.586	1	0.444
Minslastfed	0.000	1	1.000
Age	0.581	1	0.446
RH	1.304	1	0.254
RBTP:			
24hVol	0.191	1	0.662
24hKgCMR	0.035	1	0.852
Minslastfed	0.021	1	0.884
Age	0.021	1	0.884
RH	0.021	1	0.884

3.3.3.1 Body surface area

A smaller proportion of time was spent in the test pen by calves with a smaller body surface area (0.48 (48%) ± 0.042 for ≤1.15 m^2, 0.66 (66%) ± 0.034 for >1.15m^2 (mean ± se) (Figure 3.8). A body surface area greater than the median (>1.15m^2) had a significant effect on the proportion of time spent in the test pen (PropTP) (*p= 0.044*).

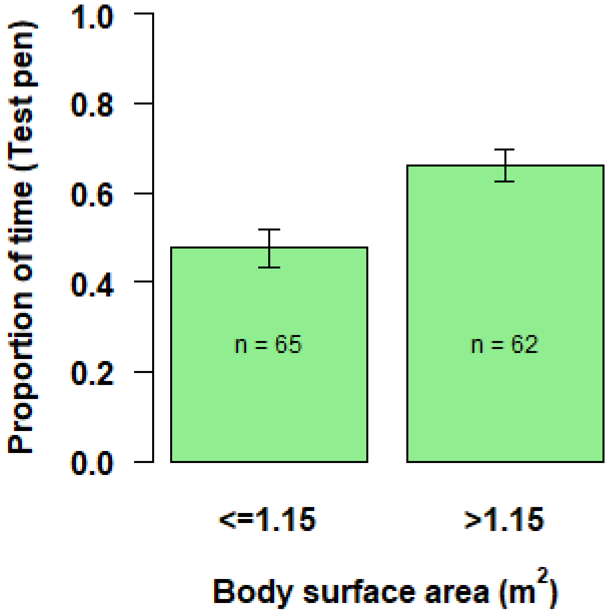


Figure 3.8 Effect of body surface area (m^2) on the proportion of time in the test pen (n= number of test sessions per category of body surface area, categorised by median)

As body surface area increased, the total number of behavioural reactions from the calves when in the test pen increased (≤1.15m^2, 10.3 behavioural reactions ±1.61; >1.15 m^2, 16.7 behavioural reactions ± 1.89 (mean ± se)) (Figure 3.9). A body surface area greater than median (1.15m^2) had a significant effect on TbehTP (*p=0.013)*. There was no significant effect of body surface area on Lat1Place (p= 0.061), Lat1React (*p= 0.663*) or RBTP (*p= 0.774*). There was also no significant effect of body surface area on the five dependent variables in the absence of wind (PropTP, p=0.901; Lat1Place, p= 0.535; TbehTP, p= 0.306; Lat1React, p= 0.631; RBTP, p= 0.836).

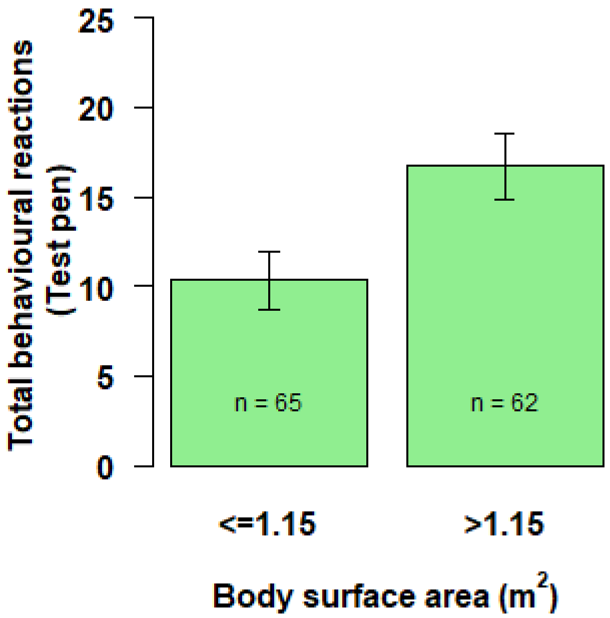


Figure 3.9 Total number of behavioural reactions in the test pen by body surface area (m^2) (n= number of test sessions per category of body surface area, categorised by median)

3.4 Discussion

The aim of this experiment was to examine the behavioural response of the pre-weaned dairy calf to different wind speeds and air temperatures considered to be around or below LCT. The hypothesis was that the calf would respond more at the higher wind speeds and lower air temperature. The calf would seek shelter quicker and display more ‘annoyance’ behaviours under these conditions than at no wind speed and the higher air temperatures.

3.4.1 Effect of wind speed

Wind speed had a significant effect on the various behavioural responses of the calves in this study. As wind speed increased, calves spent less time in the ‘test’ pen and took less time to initially move to the ‘shelter’ pen.

Therefore the calves' behavioural response was to try and remove itself from the situation (wind) by moving to the 'shelter' pen where there was no wind treatment. It was therefore seeking an area that would provide a more comfortable microclimate to minimise heat loss (Rowan, 1992). A similar concept was proposed by Houseal and Olson (1995), who found that cows tended to try and seek moderate to fully protected areas at cold temperatures along with high wind speeds. A study reported by Baldwin (1973) mentioned that pigs also avoided exposure to wind. In the current study, there was no difference between the test pen and shelter pen in terms of 'attractiveness' for the calf apart from the shelter pen not having the addition of the wind treatment. It is likely that the calf perceived the 'shelter' pen as a place of protection. Physiologically what is essentially happening in this situation is that the small layer of warm air surrounding the skin of the calf created through the process of convection is being stripped away by the wind (Carstens, 1994). Findings by Holmes and Mclean (1975) showed that there was a significant effect of wind speed on the heat produced by calves. They found that the calves produced more heat when exposed to wind speeds of 5.6km/h (1.55m/s) than when exposed to wind speeds of 0.8km/h (0.22m/s).

In this study, the calves were still observed 'removing' themselves from the situation when there was a wind speed of 0m/s as they only spent 68% of their time in the test pen and it took those 540.2s to initially move from the test pen to the shelter pen. There was no great incentive for the calf to move from the test pen to the shelter pen as both areas of the testing arena were very similar. An explanation for this maybe that calves have a limitless curiosity (Webster, 1984) and this may represent their baseline response time to explore the environment.

The results also showed that as the wind speed increased, the calves were quicker to express any of the behavioural reactions and displayed more of them. This would suggest that the calves regarded the increase in wind speed as a negative experience.

There is however a confounding issue within these results, that being the noise and visual effect of the fans. It could be said that the reactions gained were as a result of the calf being afraid of the noise generated by the fan rather than the wind that was generated. It was felt that this was not the case as Figure 3.4 shows that at 1m/s calves took 462.4s to move for the first time between the test pen and the shelter pen which was only under 80s less time to move than when they were tested at 0m/s when the fans were visible but not switched on. Therefore had the noise generated by the fans caused the reactions, then it would have been expected that the latency to first movement between the test pen and shelter pen would have been quicker.

3.4.2 Effect of air temperature

The air temperatures used in this study had no significant effect on any of the behaviour responses examined. This result might raise a few questions. Firstly, it would question if the air temperatures chosen for this study were appropriate. It was believed by the author that these temperatures were representative of air temperatures that correctly represented thermal comfort for calves (15^{o}C), lower critical temperature (10^{o}C) and a temperature where the calf would be experiencing cold (5^{o}C) as shown in the literature. If more 'extreme' air temperatures had been examined, for example -10^{o}C and +25^{o}C, then perhaps there might have been an effect of air temperature on the behavioural responses. A study by Ingram and Legge (1970) looking at the thermoregulatory behaviour of pigs in a natural environment found that the pigs did not use the shelter or huddle until the air temperature was below 5^{o}C. This study involved using calves on an individual basis and had more than one calf been used, then perhaps a similar result to Ingram and Legge (1970) may have been observed. A study by Borderas et al. (2009) showed that when given a choice, young calves had a preference for warmer environments. This result does however follow a statement made by Roe (1982) in that the general consensus is that air temperature is unimportant with regards to the optimum temperature within calf housing.

The lack of effect of air temperature on the behaviours assessed may also raise the question as to whether or not air temperature on its own is a suitable measurement in terms of environment that affect calves. Hahn (2013) states that because of the limitations of air temperature to represent the thermal environment, a combination of measures should be considered. An example of such would be the use of effective ambient temperature that combines air temperature, radiation, air movement, precipitation and humidity (Ames, 1980). This would give a more authentic representation of what the calf was experiencing. However, foundation studies surrounding the thermal regulation of calves such as Gonzalez-Jimenez and Blaxter (1962), do not take any other environmental variables into consideration, which suggests that their results do not fully represent the conditions that cause chilling in calves.

3.4.3 Body surface area

From this study it has been shown that calves with a larger body surface area spent more time in the ‘test’ pen than calves with a smaller body surface area. In contrast, calves with a larger body surface area displayed more of the reactive behaviours than calves with a smaller body surface area. This result is slightly contradictory. A possible explanation for this is that the calves with the larger body surface area have a much larger area to lose heat from and therefore it may take longer for the calf to perceive being cold as a result of vasoconstriction of the blood vessels from the extremities of the body (e.g. ears and limbs). Therefore as the calf with the larger body surface area is spending a higher proportion of its time in the test pen, then there would be more opportunity to observe more of the behavioural reactions. However, this would imply that there was a linear relationship between time spent in the test pen and the number of behavioural reactions where, as the amount of time in the test pen increased, the number of behavioural reactions increased but smaller calves had a steeper relationship function. This was not investigated. The issue surrounds why the calf did not move at the point it felt cold rather than remain in the test pen and show more of the reactive behaviours. Overall, this result remains relatively unexplained.

3.4.4 *Implications for calf management*

In this study, no calves were seen shivering in response to the environment of the testing arena and there was no deviation in core body temperature between pre and post-test session. Also, the intake and feeding behaviour related variables (the volume of reconstituted milk consumed in 24 hours prior to the test session commencing, the amount of milk replacer consumed in the same time period or the number of minutes from last milk feed until test session) were seen not to be having any significant effect on the behaviours assessed in the study. Feeding more energy is one of the suggested methods for alleviating the effects of cold stress in calves (Davis and Drackley, 1998). Gonzalez-Jimenez and Blaxter (1962) explored this concept by feeding calves either 6 litres of milk or 4 litres. They found that the resistance of cooling was less in the calves fed 6 litres than those fed 4 litres and that the lower critical temperature of the calves fed 6 litres at 3 to 4 weeks of age was 7-8^{o}C rather than 1^{o}C. Therefore, due to the mean quantity of milk replacer consumed (0.9kg ± 0.29 (mean ±sd), it could be considered that the calves were producing enough metabolic heat to keep themselves comfortable as a result of being fed what would be thought of as a high energy milk diet.

Another action for consideration would be the application of a calf jacket as a physical barrier to the effects of wind/draughts. Scoley et al. (2019) reported a 6.37^{o}C increase in the skin temperature of calves that had calf jackets compared to calves that did not receive a calf jacket when the ambient air temperature was 7.7^{o}C (mean of their study). Rawson et al. (1989) concluded that there was a 52% increase in whole animal insulation when calves wore a calf jackets at extremely low ambient temperatures. However, Earley et al. (2004) suggest that in terms of calf performance, there are no beneficial effects of having worn a calf jacket or not. Although carried out on horses and blankets instead of jackets, by the use of operant conditioning, Mejdell et al. (2019) found that the probability of the horse choosing to have a blanket on increased as wind speed increased. Therefore the horse was signalling when it perceived feeling cold.

As it has been highlighted, there are some very simple applications that can be used to alleviate the impact of cool conditions resulting from a windy environment on the calf.

3.5 Conclusion

The results of this study have shown that calves show an aversion to increasing wind speed. The study has also provided evidence that suggests calves should be housed in an area that is free from draughts. Future work might examine the behavioural response to the effective ambient temperature rather than individual measures of wind speed and air temperature alone. It may also consider extending the period of which the calf was exposed to the ‘wind effect’ and examining the effect that such conditions have on the overall performance of the calf. Other future work needed might consider incorporating operant conditioning as used in Mejdell et al. (2016) into a study of similar design to this, to further investigate the need for calf jackets under differing environmental conditions as this would help develop a best practice guide on their use in terms of what the calf requires.

Chapter 4: Effect of the housing climatic environment on the growth of calves in the first month of life

4.1 Introduction

The main objective of rearing calves for the farmer is to produce a healthy calf that is able to achieve target growth rates as economically as possible. It has been well documented that poor performance in an individual calf in the pre-weaning phase can affect its future productivity (Bach 2011; Soberon et al., 2012; Soberon and Van Amburgh, 2013; Van De Stroet et al., 2016; Chester-Jones et al., 2017). Therefore, calf growth, specifically daily liveweight gain (DLWG) is a key performance indicator for monitoring success in calf rearing. DLWG is influenced by colostrum provision (Robison et al., 1988; Godden, 2008), on-going nutrition, incidents of disease, and by the quality of the calf's environment (Place et al., 1998; Sherwin et al., 2016).

The introduction to the previous chapter (Chapter 3) informed that following the birthing process calves are exposed to an environmental temperature that is typically below their TCZ and also as previously mentioned the calf's environment can influence its growth.

Studies have investigated the effect of temperature on growth in calves. Cockram and Rowan (1989) carried out a study using calves less than four weeks of age in controlled environmental chambers of 10^{o}C and 25^{o}C. They found that calves housed within the chambers at 10^{o}C had lower liveweight gains at 17-22 days of age than the calves housed in the chambers at 25^{o}C. Broucek et al. (2009) reported that there was only a slight decrease in DLWG in calves that were exposed to high temperatures (average of 26.5^{o}C) compared to those exposed to temperatures regarded by the author as moderate (averages of 19.5^{o}C and 15.7^{o}C). These high temperatures were recorded between June and September which are regarded as summer months. Additionally, various studies have indicated that season of birth can influence DLWG in calves. Place et al. (1998) found from their study conducted in Pennsylvania, US, that there was a tendency for calves born in winter to have higher average DLWG than calves born in the other seasons of the year. This result was also found from the study carried out in

Minnesota, US by Chester-Jones et al. (2017) where calves born in the autumn and winter gained 0.66kg/d compared to 0.62kg/d in calves born in summer. Therefore, as previously alluded to season of birth and temperature are slightly confounding as lower temperatures would be expected during the winter months and higher temperatures during the summer months. As well as temperature and season of birth, Kelly et al. (1984) highlighted the effect of relative humidity levels on DLWG. They examined the DLWG of calves housed at two temperatures (7^{o}C and 15^{o}C) and two levels of relative humidity (75% and 95%) and found that there was no difference in the DLWG between the relative humidity levels when housed at 15^{o}C. However, there was a significant increase ($p<0.05$) in DLWG when housed at 7^{o}C with 75% relative humidity compared to 7^{o}C with 95% relative humidity levels. Therefore, this shows that there is more than one aspect of the thermal environment that can influence DLWG.

Nutrition, in particular milk consumption, can also influence DLWG. A study by Rosenberger et al. (2017) showed that giving calves a higher milk volume allowance resulted in a higher weight gain. However, a coping mechanism for calves that are exposed to temperatures below the LCT is to increase feed consumption if additional feed is available (Baldwin, 1973). There is also evidence to show that nutrition and the environmental temperature can affect daily liveweight gain (Cockram and Rowan, 1989) and therefore it is generally advised that calves are provided with more energy when the environmental temperature falls below the LCT (National Research Council, 2001; Davis and Drackley, 1998) in order to allow the calf to partition some of this energy towards growth as well as heat production required for maintenance. This increase in energy can be achieved by methods such as increasing the volume of milk offered or quantity of calf milk replacer (CMR) fed to the calf. The practice of feeding calves milk replacer is common in the UK (Cooper and Watson, 2013) as it is a consistent product in terms of fat, protein and energy content, in contrast to whole milk. There have been various studies that have examined the quantity of CMR fed and the effect it has on DLWG. Morrison et al. (2009) found that calves offered 10l/d (1200g

CMR/d) had significantly higher growth rates until weaning than calves offered 5l/d (600g CMR/d) and the similar finding of increasing the quantity of CMR fed having a significant effect on DLWG was found in a later study (Morrison et al., 2012). Quigley et al. (2006) also reported that calves fed a larger quantity of calf milk replacer (CMR) had better feed efficiency and a higher liveweight gain but had a higher incidence of calf diarrhoea as well.

Studies such as Cockram and Rowan (1989) have been conducted under controlled environmental conditions or where the management of the calves has been adjusted. The objective of this longitudinal observational study was to conduct such a study using naturally occurring environmental conditions with the same management regime throughout. As an alternative to using air temperature, this study aimed to examine the effect of the proportion of hours that calves were exposed to effective temperatures below their LCT on their DLWG for two management phases; the first days of life when the calf is in an individual hutch (phase 1), and then when the calves are moved into group pens (phase 2).

4.2 Materials and methods

This study was conducted between July 2018 and July 2019 at the SRUC Dairy Research & Innovation Centre, Crichton Royal Farm, Dumfries (Home Office PPL P204B097E, SRUC animal experiment research project ED AE 21-2018).

4.2.1 Calf housing and management

The calves used in this study were sourced from the dairy herds at SRUC Dairy Research & Innovation Centre and their management followed normal farm management practices. All calves born within the study period were eligible for recruitment onto the study and consisted of dairy (Holstein) and dairy-beef cross (Holstein-British Blue cross, Holstein-Limousin cross, Holstein-Aberdeen Angus cross). Both male and female calves were eligible for recruitment.

All calves were removed from their dam within 24 hours of birth and given an identification eartag, had their navel dipped with strong iodine solution, oesophageal tubed with four litres of thawed quality tested pasteurised colostrum and weighed (birthweight). The calves were then taken to the main calf rearing unit and placed within a straw bedded individual calf hutch (Calf-Tel Compact, *Calf-Tel, Hammel Corporation, Wisconsin, USA*) where they had access to fresh water and ad-lib starter pellets (*VitaStart + Deccox, ForFarmers, Suffolk, England; crude protein 18%, crude fats & oils 4%, crude fibre 11.5%, crude ash 7%, 3mm diameter*) daily via buckets. From this point on, the calves received three litres of reconstituted milk replacer (*Omega Gold, ForFarmers, Suffolk, England*) twice a day (0730h and 1600h approximately) via teat buckets. The milk replacer consisted of 23% crude protein, 18% crude fat, 0.1% crude fibre and 8.5% crude ash and was fed at a concentration of 15%. All the buckets for each calf remained with that calf for the duration of time it remained in the individual calf hutch. As a matter of routine, all calves received 4ml Halocur ® (*Intervet International, The Netherlands*) for the first six days of life due to the history of cryptosporidiosis on the farm. Once calves were able to suckle from the teat confidently and were assessed to be strong and healthy enough, the calves were eligible to move into the group housing Igloo system.

The Igloo system consisted of a hand laminated fibre-glass constructed dome (Igloo) (*Holm & Laue, Westerfield, Germany*) (3.9m, 4.4m, 2.2m: length, width, height respectively, volume $20m^3$). In front of the Igloo there was a roofed pen (5.1m x 5.1m – length & width). The flooring of both areas was covered in straw. New straw was added weekly and all bedding was removed and replaced every 2 weeks. The main calf rearing unit consisted of 2 rows of 4 group Igloo pens (Figure 4.1). Every pen contained an automatic milk feeder from which every calf was allowed up to 7.2l (7l plus

0.2l carry-over allowance) of reconstituted milk replacer every day. This was fed at a concentration of 15%.

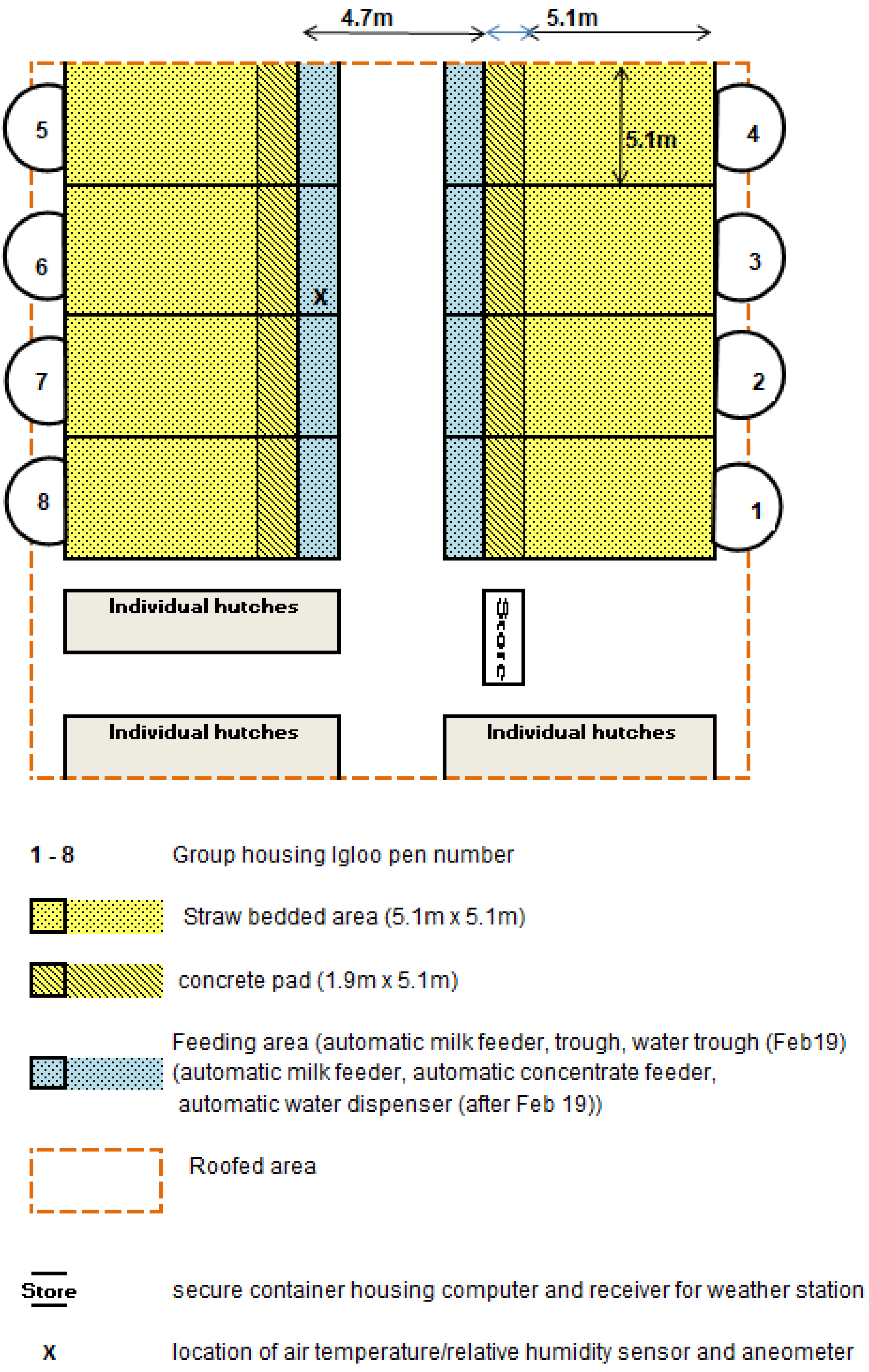


Figure 4.1 Diagram of main calf shed

Upon leaving the individual hutch and entering the group housing Igloo system, every calf received 2ml Rispoval® RS + PI3 IntraNasal (*Zoetis, Belgium*). It was normal practice for the farm to move calves into the group housing Igloo pen between 6 and 14 days of age.

4.2.2 Climate data

Air temperature (^oC), relative humidity (%) and wind speed (m/s) were automatically recorded hourly throughout the study period using a Ventus W831 Weather Station (*NSH NORDIC A / S The, field 4, DK-8740 Brædstrup*). A sensor to measure air temperature and relative humidity and the anemometer for the measurement of wind speed were located in the central passage of the calf shed at 0.8m and 1.5m respectively. The data from both sensors were downloaded twice per week.

4.2.3 Measurements

For this study, measurements were taken for two management phases. Phase 1 covered the period from when the calves went into the individual hutch until they left it and went into the group housing Igloo pen. This phase is referred to as '*B2G*' hereafter. Phase 2 covered the period from when the calves entered the group housing Igloo pen until the end of the study. This phase is referred to as '*G2E*' hereafter.

Calves were enrolled onto the study when they left the individual hutch and entered the group housing Igloo pen. Data on date of birth, birthweight (kg), calving ease and parity of dam were collected retrospectively from farm records. The calf's date of birth was used to define its season of birth (winter (December, January, February), spring (March, April, May), summer (June, July, August), autumn (September, October, November)). Data on air temperature (^oC), wind speed (m/s), and relative humidity (%) for the period when the calf went into the individual hutch until it left the individual hutch was also collected retrospectively from the weather station in the calf shed.

4.2.3.1 Individual hutch (B2G)

Calves were weighed when they were removed from their dam using a manually operated calibrated weigh crate with Tru-Test digital load cells and EziWeigh5 weigh head attached (*Ritchie Agriculture, Forfar, Angus, Scotland*). This weight was referenced as 'birth weight'. It was not noted whether or not the calf was weighed after or prior to colostrum consumption. The calves were weighed again using the same weigh crate, at the point of leaving the individual hutch and entering the study (*LH* weight). Daily liveweight gain (DLWG) (kg/d) for *B2G* was calculated by dividing the weight gain by the number of days between birth weight and entry weight. Calf level treatments were collected from farm records, and calves were classified as either having received treatment or not during this period (*No, Yes*).

4.2.3.2 Group housing Igloo pen (G2E)

Whilst in the group housing Igloo pen, there were two recording days per week, typically Monday and Thursday (1230h – 1400h), and occasionally on other days where circumstances intervened. Various measurements were taken from each of the calves on these days.

4.2.3.2.1 Liveweight

A record of liveweight (kg) was taken for each calf on the recording days and this weight was assigned a weighing number to represent whether this was the calf's first, second, third etc. recording of liveweight whilst in the group housing Igloo pen for the duration of the study (WGT1, WGT2, WGT3, WGT4, WGT5, WGT6, WGT7). Daily liveweight gain (DLWG) (kg/d) for *G2E* was calculated but, to take account for the possible changing rate of DLWG through time between entering to the group housing and end of study period, a linear regression was applied for *G2E* with the value of the slope used as DLWG (Tolley et al., 1988; Sherwin et al., 2016).

4.2.3.2.2 Health scoring

A health assessment of each calf was made on the day the calf left the individual hutch and on each recording day using the Wisconsin method (McGuirk, 2008; McGuirk and Peek, 2014). This was carried out by the same trained operator. This method involved taking rectal temperature, visually assessing ocular and nasal discharge, head/ear positioning and the presence or absence of a cough. Each aspect was given a score on a scale of 0 to 3 with 0 being described as 'normal' and 3 as 'severe'. An accumulation of these scores represented the overall health score for the calf with the lowest possible score being 0 and the maximum score of 15. For this study, rectal temperature was taken using a digital thermometer (*Genia Digiflash, St. Hilaire de Chaléons, France*). A score for faecal consistency was not able to be carried out as the calves were group-housed and faeces from individuals could not be identified, so was not included in the analyses of health status.

From the scoring, calves were defined as either 'healthy', 'diseased' or 'intermediate' based on the criteria in Table 4.1.

Table 4.1 Calf health status definitions based on Wisconsin health scoring method (McGuirk, 2008)

Health status	Signs of Disease	Definition criteria
Healthy	No	Rectal temperature score less than or equal to 2 with an overall Wisconsin score of equal to or less than 3
Intermediate	Yes	Rectal temperature score less than or equal to 2 with an overall Wisconsin score equal to 4
Diseased	Yes	Rectal temperature score equal to 3 regardless of overall health score; Rectal temperature score less than 3 with an overall Wisconsin score of equal to or greater than 5

Health status for the calf was then categorised for the *G2E* period of the study into ‘ever showed clinical or mild signs of disease’ (Intermediate and Diseased – signs of disease, Yes) or ‘never showed any signs of disease’ (Healthy – signs of disease, No).

Farmstaff who were involved in the care of the calves were made aware of the results of the health assessments and treatment was administered at the discretion of the farm. Calf level treatments were collected from farm records, and calves were classified as either having received treatment or not during this period of the study (*No, Yes*).

4.2.3.2.3 Milk intake data

Milk consumption data (quantity of calf milk replacer (CMR) consumed each day) was collected for each calf for the period it was in the study (from entering the group Igloo pen to the last time it was weighed). The milk feeding equipment was changed within the study period. Initially calves were fed from an H&L100 (*Holm & Laue, Westerfield, Germany* – 183 calves) and latterly from a BioControl milk feeder (*BioControl AS, Grimstad Gård, N-1890 Rakkestad, Norway* – 116 calves). No calf was fed using both milk-feeding systems.

The number of days from the calf entering the group pen until the last day of measurements being taken was calculated and used to determine the average daily intake of milk (l/d) and CMR (g/d) for each calf. Only average daily CMR intake was used in the analysis.

4.2.4 Data analysis

4.2.4.1 Proportion of hours below LCT

Based on the hourly measurements taken from the weather station, the effective temperature (^oC) for every hour was calculated using the *etv* function from the '*ThermIndex*' package in R (Castelhano, 2017). This function incorporates air temperature, relative humidity and wind speed and has been based on the equation by Suping et al. (1992):

$$ET_v = 37 - (37 - ta) / [0.68 - 0.0014RH + 1/(1.76 + 1.4v^{0.75})] - 0.29ta\,(1 - RH/100)$$

where, ET_v represents effective temperature with wind speed (^oC), *ta* is air temperature (^oC), *RH* is relative humidity (%) and *v* represents wind speed (m/s).

Effective temperature was chosen, as although air temperature is widely used to assess the thermal conditions, other parameters such as relative humidity and wind speed can amplify the perception of high and low temperatures (Roland et al. 2016).

The lower critical temperature (LCT) was calculated per day per calf from birth (day 0) to the day they left the study. LCT at birth (day 0) was defined as 15^oC (Stull and Reynolds, 2008) and decreased with the age of the calf (Davis and Drackley, 1998) by 0.5^oC per day.

The proportion of total hours that each calf experienced an effective temperature below this LCT was then calculated. This was done by calculating the number of hours the effective temperature was below that of the associated age related LCT for each calf and then dividing that by the total number of hours the calf was in each phase of the study (individual hutch (*B2G*) and group Igloo pen (*G2E*)).

The proportion of hours below LCT was then categorised into quartiles; *B2G* (≤0.32, 0.33 – 0.58, 0.59 – 0.96, ≥0.97), *G2E* (≤0.01, 0.02 – 0.06, 0.07 – 0.27, ≥0.28).

4.2.4.2 Statistical analysis

All statistical analyses were conducted using R-software (R Core Team, 2016). Datasets were compiled for the two time periods of interest for the study: birth to group pen entry (*B2G*) and group pen entry until the end of the study period (*G2E*).

For each calf, data on variables (Table 4.2) were recorded and included in the appropriate dataset.

Any calves missing a birth weight were excluded as DLWG could not be calculated. As it was normal practice for the farm to move calves into the group housing Igloo pen between 6 and 14 days, calves older than 14 days were excluded. Other reasons for exclusion included the sale or death of the calf before the end of the study period, the return of the calf into the individual hutch after initially entering the group housing Igloo pen, milk consumption data being unavailable and only having one weight in the group housing Igloo pen. The dependent variable, DLWG, was investigated for association with the independent variables mentioned in Table 4.2.

To analyse the dataset for the time period of *B2G*, multiple univariable linear models using the *lm* function were used to screen the independent variables.

To analyse the dataset of *G2E*, multiple univariable linear mixed effect models with random effects for the group in which the calf was placed, the group Igloo pen in which they were placed and the milk feeding system used were constructed. The *lmer* function from the '*lme4'* package (Bates et al, 2015) was used. For both sets of analyses, independent variables that had a P value of less than 0.20 were carried forward and included in a multivariable model.

A maximal model was constructed and then optimised using backward step selection until only variables significant at $p<0.05$ remained. Post hoc Tukey tests were used to obtain significance between factor levels of significant variables only.

Table 4.2 Inclusion of variable of interest in the two datasets

Variable	*B2G*	*G2E*
Sex of calf (*male, female*)	✓	✓
Breed classification (*Dairy, Dairy-Beef x*)	✓	✓
Calving ease (*assisted birth, unassisted birth)*	✓	✓
Parity of dam at birth (*primiparous, multiparous)*	✓	✓
Season of birth (*winter, spring, summer, autumn)*	✓	✓
Birth weight (kg)	✓	✓
Treatment administered (Farm) – Hutch (No, Yes)	✓	✓
Age at leaving individual hutch/entering group housing Igloo pen (d)	✓	✓
Treatment administered (Farm) – Igloo (No, Yes)		✓
Signs of disease (based on Wisconsin score) (*No, Yes*)		✓
Average quantity of CMR consumed (g/d)		✓
Proportion of hours below LCT (effective temperature)	✓	✓
Daily liveweight gain (kg/d)	✓	✓

4.3 Results

4.3.1 Calves – descriptive statistics

In total, 299 calves were enrolled onto the study. Of these, 226 were dairy calves (137 female, 89 male) and 73 were dairy-beef cross calves (34 female, 39 male). Of these 299 calves, 109 were born from primiparous dams and 190 from multiparous dams. In terms of calving ease, 263 had an unassisted birth and 36 had an assisted birth. Out of the 299 calves, 80 were born in winter (26 dairy, 54 dairy-beef cross), 85 in spring (76 dairy, 9 dairy-beef cross), 54 in summer (45 dairy, 9 dairy-beef cross) and 80 in autumn (51 dairy, 29 dairy-beef cross).

One calf was excluded from the data due to a missing birth weight and 27 were excluded as they were older than 14 days when moved into the group housing Igloo pen. Data from the remaining 271 calves was used in the analysis of the dataset for *B2G*.

In terms of the age at which these 271 calves left the individual hutch and entered the group housing Igloo pen, 19 calves were 6d, 52 calves were 7d, 45 calves were 8d, 45 calves were 9d, 29 calves were 10d, 28 calves were 11 d, 27 calves were 12d, 16 calves were 13d and 10 calves were 14d.

Of the 271 calves, 37 received treatment by the farm whilst in the hutches (19 diarrhoea (6.1d ±2.05), 13 respiratory disease (6.1d ± 2.36) and 5 other disease (3.6d ±1.63) (mean age at treatment ±sd)).

Five calves died or were euthanased whilst on study (one experienced a seizure, two suffered traumatic leg injuries, one had an umbilical abscess, and one had an injury to an eye that was not amenable to treatment) and one calf was sold before completing the study. Three calves were also excluded as they returned to the individual hutches from their group Igloo pen. There were two separate occasions throughout the study where milk feeding data was unable to be recovered as a result of power failure. This affected 41 calves which were excluded from this study. Following exclusions, the dataset used for the analysis for the *G2E* time period contained 221 calves.

It can be seen from Table 4.3 that for the time period *B2G*, the calves on average, lost weight (mean DLWG -0.07kg/d), although there was a large variation in DLWG. This variation was slightly less for *G2E*. For the period *G2E*, 98 calves showed no signs of disease (all scores in Healthy category) and 123 showed signs of disease (one or more score in the Intermediate and Disease category).

4.3.2 Climate – descriptive statistics

Over the course of the study, there was a range of climatic conditions experienced (Table 4.4). For the time period from birth until leaving the individual hutch (*B2G*) some calves experienced an effective temperature that was always below their LCT related to age whereas others did not. On average, calves in the individual hutch spent 60% of the time below their LCT.

4.3.3 Daily liveweight gain - Birth to group pen (B2G)

The final multivariable linear model for daily liveweight gain of calves from birth until they left the individual hutch and entered the group housing Igloo pen (*B2G*) contained the variables: the proportion of hours the calf was exposed to effective temperatures below age related LCT (ProphrsLCT), weight of calf at birth (birth weight) and the age of the calf at which it left the individual hutch (Age leaving individual hutch) (Table 4.5). No other variables were significant in the model.

There was a significant effect of ProphrsLCT on DLWG (*B2G*) ($F(3, 265) = 6.098$, $p<0.001$). There was a significant difference found between the categories ≤0.32 and 0.59 – 0.96 ($p=0.015$), ≤0.32 and ≥0.97 ($p<0.001$) and between 0.33 – 0.58 and ≥0.97 ($p=0.041$) (Figure 4.2). When the calf experienced over 58% of its time below the LCT, there was a reduction in DLWG with the effect being stronger when the calf experienced over 97% of its time below the LCT.

Table 4.3 Description of calf related parameters

Phase/Parameter	Mean	SD	Median	Minimum	Maximum
B2G					
Birth weight (kg)	43.2	6.2	42.0	31.0	67.0
LH weight (kg)	42.7	5.8	42.0	29.0	65.0
Age leaving individual hutch (d)	9.3	2.2	9.0	6.0	14.0
DLWG(kg/d)	-0.07	0.34	-0.08	-1.33	1.00
G2E					
Birth weight (kg)	43.3	6.2	42.0	31.0	67.0
Group pen entry age (d)	9.1	2.2	9.0	6.0	14.0
Entry weight (kg)	42.9	5.9	42.0	29.0	65.0
End weight (kg)	55.1	7.3	54.0	38	78
End Age (d)	29.5	1.2	30.0	25.0	32.0
DLWG (kg/d)	0.60	0.20	0.60	0.00	1.00
Average CMR intake (g/d)	890.5	152.3	909.6	489.8	1223.7

LH weight – weight upon leaving individual hutch, DLWG – daily liveweight gain

Table 4.4 Description of climate parameters

Parameter	Mean	SD	Median	Minimum	Maximum
[1] Air temperature (^{o}C)	10.3	5.2	10.0	-3.9	26.7
[1] Wind speed (m/s)	0.2	0.4	0.0	0.0	3.0
[1] Relative humidity (%)	81.1	11.3	84.0	27.0	99.0
[1] Effective temperature (^{o}C)	11.3	5.1	11.3	-5.3	24.2
[2] Proportion of hours effective temperature below LCT (*B2G*)	0.60	0.33	0.58	0.00	1.00
[3] Proportion of hours effective temperature below LCT (*G2E*)	0.15	0.17	0.06	0.00	0.61

[1] *Based on data from the day the 1st calf recruited to the study was born until the day the last calf was weighed on study)*

[2] *Based on data for 271 calves*

[3] *Based on data for 221 calves*

Table 4.5 Final model describing variables affecting daily liveweight gain from birth until entering the group pen (*B2G*)

	Daily liveweight gain (kg/d) *B2G*				
Variable	Level	Estimate	SE of estimate	P value for reference	P value for effect
Intercept	-	0.537	0.153	-	<0.001
ProphrsLCT	≤0.32	*Reference*	-	-	<0.001
	0.33 – 0.58	-0.061	0.052	0.240	
	0.59 – 0.96	-0.157	0.052	0.003	
	≥0.97	-0.199	0.052	<0.001	
Birth weight (kg)	-	-0.018	0.003	-	<0.001
Age leaving hutch (d)	-	0.028	0.009	-	<0.001

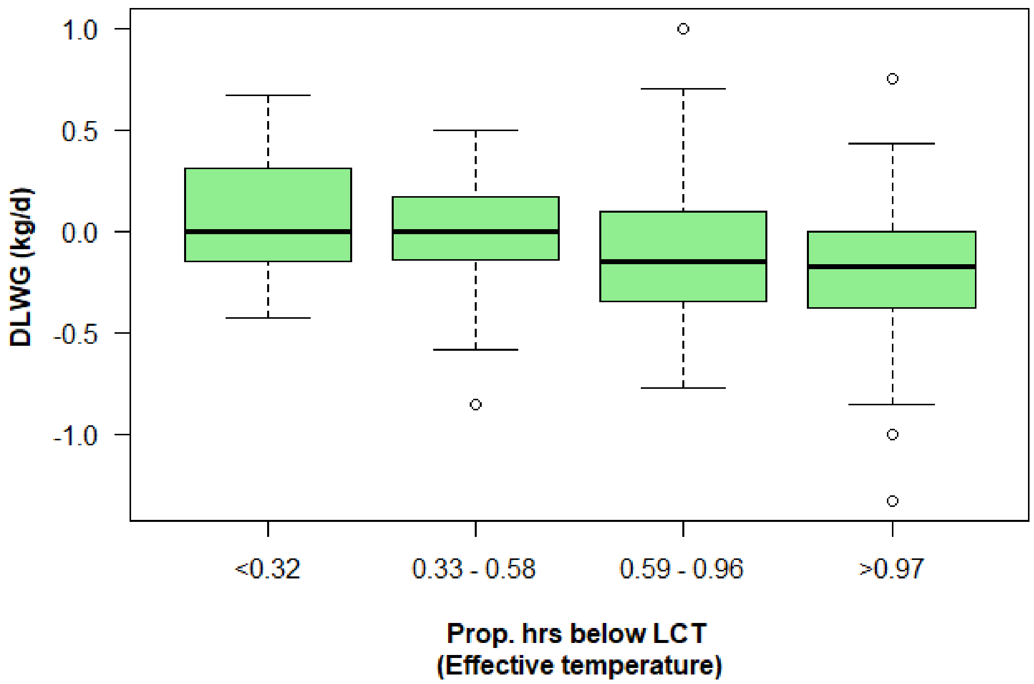


Figure 4.2 Effect of proportion of hours below LCT on daily liveweight gain (DLWG, kg/d) for the period between birth and entering the group housing Igloo (*B2G*)

The birth weight of the calf had a significant effect on DLWG for the period between birth and leaving the individual hutch (F (1,265) = 35.154, $p<0.001$). When accounting for all other variables in the model, for every Kg increase in birth weight, DLWG (*B2G*) reduced by 0.02kg/d.

The age at which the calf left the individual hutch also had a significant effect on the daily liveweight gain (DLWG, kg/d) (F $(1,265_{)}$ = 11.196, $p<0.001$). DLWG (*B2G*) increased by 0.03kg/d for every day older the calf was when leaving the individual hutch.

4.3.4 Daily liveweight gain - Group pen until end of study period (G2E)

The final model examining factors affecting the daily liveweight gain of calves from entering the group housing Igloo pen until the end of the study period (*G2E*) contained the proportion of hours the calf was exposed to effective temperatures below age related LCT (ProphrsLCT), age of the calf at entry to the group pen (Entry age) and their average daily intake of CMR (CMR intake) (Table 4.6).

Table 4.6 Final mixed model describing factors affecting daily liveweight gain (*G2E*)

	Daily liveweight gain (kg/d) *G2E*				
Variable	Level	Estimate	SE of estimate	P value for reference	P value for effect
Intercept	-	-0.301	0.065	-	<0.001
ProphrsLCT	≤0.01	*Reference*	-	-	0.432
	0.02 – 0.06	-0.037	0.025	0.132	
	0.07 – 0.27	-0.008	0.027	0.776	
	≥0.28	0.002	0.031	0.958	
Entry age (d)	-	0.009	0.004	-	0.012
CMR intake(g/d)	-	0.001	0.000	-	<0.001

There was no significant effect of ProphrsLCT on daily liveweight gain (*G2E*) (*chisq* = 2.747, 3 df, *p=0.432*).

The age at which calves entered the group housing Igloo pen also had a significant effect on DLWG for *G2E* (*chisq* = 6.343, 1 df, *p=0.012*). The final model indicated that for every day older the calf was on entry to the group pen their DLWG (*G2E*) increased by 0.01kg/d.

There was a significant effect of the average CMR intake on the daily liveweight gain for *G2E* (*chisq* = 348.686, 1df, *p<0.001*). Also, there was a significant positive correlation between average CMR intake and DLWG (*G2E*) (r = 0.77, *p<0.001*) (Figure 4.3). The more CMR consumed by the calf, the higher the growth rate achieved.

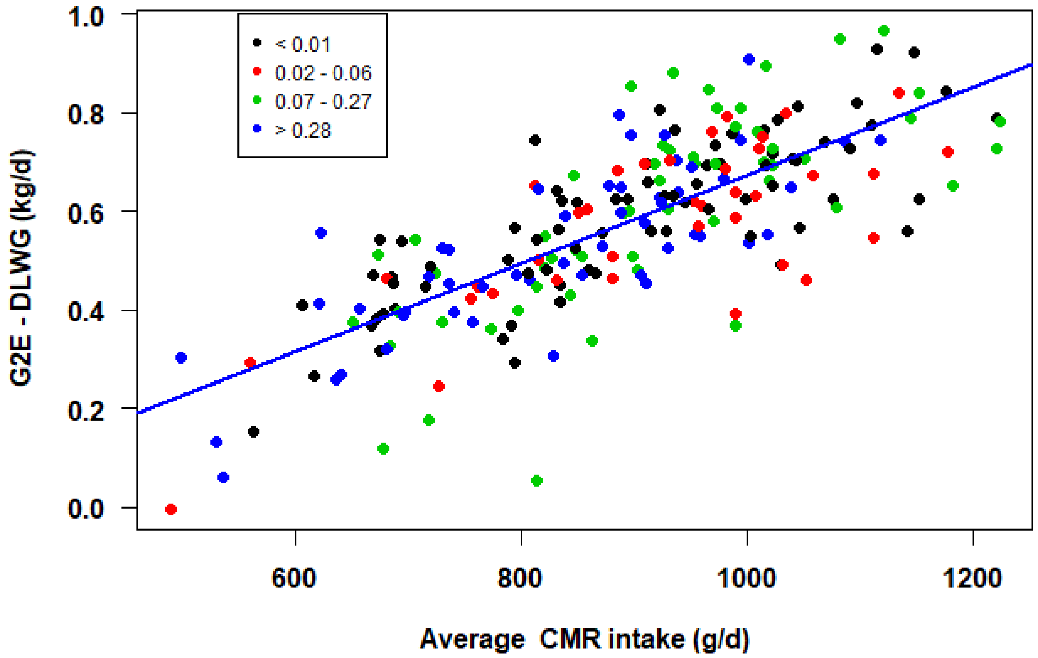


Figure 4.3 Association of average CMR intake (g/d) on daily liveweight gain (DLWG, kg/d) between entering the group housing Igloo pen and end of study period (*G2E*) by proportion of hours below LCT

4.4 Discussion

The DLWG measured for the calves in the study were in the region of that of other UK studies. Bazeley et al. (2016) reported that there was no weight gain in calves in the first eight days of life. The present study followed that trend by having a mean DLWG for *B2G* (from birth until leaving the individual hutch) of -0.07kg/d. For the time period *G2E* (from entering the group housing Igloo pen until the end of the study), the mean DLWG was 0.60kg/d for calves 29 days of age (mean age at end of study). The DLWG for the present study were slightly higher than those reported for the top performing herds in Bazeley et al. (2016) (0.52kg/d) and the non-jacketed calves in the study of Scoley et al. (2019). Differences in the housing and management systems on each of the farms in these studies will likely account for the differences in the growth rates.

However, for the B2G period there is a potential source of error with the recording of the liveweight at birth and then when the calf left the individual hutch and entered the group housing Igloo pen and subsequently the daily liveweight gain. It was not noted whether or not the birth weight was taken pre or post colostrum feeding and this may influence the body weight of the calf. Also the weight at which the calf left the individual hutch, although taken before the calf had drank upon entry to the group housing Igloo pen, the calf may or may not have defecated, urinated or consumed water or starter pellets beforehand.

4.4.1 Proportion of hours below LCT

In the present study, there was a significant effect of the proportion of hours the calves were exposed to effective temperatures below their LCT on their daily liveweight gain (DLWG, kg/d) when in the individual hutch from birth until leaving the individual hutch and entering the group housing Igloo pen. However, there was no significant effect on DLWG for the time period from entering the group housing Igloo pen until the end of the study (~28 days of age).

The proportion of hours that the calves were below their LCT was based on environmental data collected from the central passage of the calf building rather than from within each individual hutch or group housing Igloo. Therefore this could be seen as a source of error since the data will not be an exact reflection of the environment that each calf was exposed to. To successfully achieve a true reflection of the environment that each calf was experiencing, sensors would be needed to be attached to the calves to record this data. The sensors would also need to be able to locate the calf (e.g. within individual hutch, outside hutch, within group Igloo, outside in the straw area of group housing Igloo pen) as well as identify the posture of the calf (e.g. standing or lying) as Duthie et al. (2019) has shown that healthy calves can lie for over 70% of the time.

4.4.1.1 B2G period

For the period *B2G*, it is likely that the young calf is struggling to acclimatise to the environment it is kept in. According to Nienaber and Hahn (2007) this process can take days or even weeks to be achieved. Rowan (1992) reported that acclimatisation develops with the age of the animal. Another possible explanation for this result could lie within the calculation used for the proportion of hours below LCT. As mentioned in section 4.2.4.1 (Materials and methods), for each calf day, the LCT used was relevant to age based on the criterion that at Day 0 (birth) the LCT for the calf was 15^{o}C and thereafter, the LCT reduced by 0.5^{o}C each day. There would be very few calves who were exposed to effective temperatures that were above their age related LCT on an hourly basis for the individual hutch period. Only under exceptional weather in the UK would calves be exposed to effective temperatures day and night that were above their age-related LCT. Most calves in the UK, and those in the present study, would be exposed to effective temperatures at some stage during the day, but mostly at night, that were below this LCT. As this study has demonstrated, calves that were exposed to a high proportion of hours below this LCT had significantly lower DLWG. The calves were offered what could be classed as a high volume energy dense milk diet during this management stage (*B2G*) (6l/d at 15% concentration which equated to 900g CMR/d) regardless of season of birth. Therefore, despite this, the climatic environment still had an impact on the performance of these calves. However, milk intake was not recorded during this management stage and it was assumed that calves drank all the milk that was offered to them at both feedings. Therefore, this result suggests that the period from birth is when the calf is extremely vulnerable to the climatic environment and management procedures such as application of calf jackets could potentially be beneficial by acting as a barrier for reducing heat loss to the environment (Rutherford et al., 2020).

4.4.1.2 G2E period

The non-significant result for the time period from entering the group housing Igloo pen until the end of the study period (*G2E*) could also be as a consequence of the calves' development as by this stage their LCT is below the average climatic conditions for the area. The calves in the present study entered the group housing Igloo pen when their age-related LCT was 10^{o}C (mean entry age was 10 days). Every day after this, the LCT declined by 0.5^{o}C and therefore there would be a higher chance that the calf would not be below its LCT unless there had been a sudden dramatic change in environmental conditions, especially in Southern Scotland where mean daily air temperature has been estimated to be around 9^{o}C (Met Office, 2016). Another reason is related to the behavioural response of the calves. For the time period *G2E*, the calves were group housed whereas for *B2G* they were housed individually. A behavioural response to low environmental temperatures is huddling (Baldwin, 1973). Once in the group housing Igloo pen, calves had the opportunity to keep warm by huddling with the other calves in the group whereas in the individual hutch, the calf was relying on other processes such as the ability to nest in the bedding material to maintain warmth. Although not recorded in the present study, it would have been of interest to see if this behavioural response (huddling) was occurring and in particular if it occurred during specific times of the day (e.g. around dawn and overnight) and its relationship with the climatic environment.

The proportion of hours below LCT related to age and/or effective temperatures are not the conventional climatic environment parameters used to assess thermal conditions. In some literature, the Temperature-Humidity Index (THI) has been used. Shivley et al. (2018) found that calves exposed to a THI of less than 50 during the pre-weaning period had a higher DLWG compared to calves exposed to a THI between 50 and 59 and greater than 70. However, it is suggested that the THI is more of an indicator of heat stress rather than of general conditions in cooler climates. Also, the THI is based solely on a combination of air temperature and relative humidity. It does not take wind speed into consideration which effective temperature does. The housing for the calves in the present study was within an open sided umbrella-like structure and therefore the calves had the potential to be exposed to wind and therefore the use of the effective temperature was thought to be most appropriate. As Hahn et al. (2013) states, there are limitations to the use of air temperature as a representation of the thermal environment and a combination of parameters should be used. Some work has been carried out to look at the appropriateness of human comfort indexes for use in livestock, particularly heat stress (Kovács et al., 2018) but further work should be done to develop a more general index for livestock, in particular for calves.

4.4.2 Birth weight

It was found in the present study that birth weight had a significant effect on DLWG for the period from birth until leaving the individual hutch (*B2G)*. Donovan et al. (1998) and Yaylak et al. (2015) also both found that birth weight had a significant influence on subsequent daily liveweight gain. However, daily liveweight gain for both these studies were recorded for longer periods of time than in the present study (birth to 6 months and birth to weaning respectively). This effect of birth weight on subsequent growth rate may be a result of the volume of milk given to the calves during this early phase of life. When they were housed in the individual hutch *(B2G*) calves in the present study were given 6l/d of milk. In the case of the lightest calf birth weight (31kg) this equated to nearly 20% of bodyweight, whereas for the heaviest calf (67kg), the 6l/d only equated to 8.9% of bodyweight. Therefore lighter born calves potentially had more available energy, over and above that which was required for maintenance which could be used for growth. This explanation is supported by the results of Khan (2011) who showed that calves from 3d of age can safely consume 20% of their bodyweight per day and that an increase in consumption supports an increase in DLWG.

4.4.3 Age leaving individual hutch and entering group housing Igloo pen

The age at which the calf left the individual hutch and entered the group housing Igloo pen had a significant effect on DLWG in both the *B2G* and *G2E* periods. For the *B2G* time period, the older the calf was upon leaving the individual hutch and entering the group pen, the higher the DLWG. The same trend was evident for the *G2E* time period, with the greater the age at entry to the group pen, the higher the DLWG. A factor that could be influencing this result is the use of the automated feeders and the possibility that older calves take less time to learn to use them. Fujiwara et al. (2014) reported that calves that take a considerable time to learn and adapt to the automated feeders had reduced milk intake and poor growth rate in the initial weeks of entering the group pen. They also reported that calves introduced around 6 days of age took longer to voluntarily consume milk from the automated feeders than calves at 9 days of age. This result was also found from the study by Jensen (2007).

Although the age at entering the group housing Igloo pen was statistically significant for the analysis of DLWG (*B2G*) and DLWG (*G2E*), the magnitude of the result was not particularly biologically significant. The result from the final model showed that for every one unit increase in entry age the DLWG (*G2E*) increased by nine grams per day (9g/d) when experimental group, group housing Igloo pen and milk feeding system were taken into consideration. It is felt that such an increase is considered not biologically significant.

4.4.4 CMR intake

The amount of CMR consumed per day had a significant effect on DLWG for the *G2E* time period. This result concurs with those found in studies by Quigley et al. (2006), Morrison et al. (2012), Soberon et al. (2012) and Johnson et al. (2017). Morrison et al. (2012) showed that an increasing level of milk replacer produced a higher DLWG between day 0 and 28 of age and Johnson et al. (2017) found that there was a positive correlation of CMR intake with daily liveweight gain. The study by Khan et al. (2011) demonstrated that calves can safely be fed 20% of their bodyweight per day which can result in an increase in DLWG. These results could be due to the extra energy that the increase in CMR intake is providing above maintenance energy. Future work would consider examining the proportion of maintenance energy provided from the daily CMR intake in terms of above and below that which is required by the calf to validate this theory.

4.5 Conclusion

The present study has shown that the key performance indicator of daily liveweight gain is affected by the housing environment in the very early stages of the rearing phase of the calf. Therefore, emphasis should be placed upon the management of the calf at this stage and perhaps apply calf jackets, especially in times when temperatures are below the LCT. An appropriate plane of nutrition will also assist with achieving target daily liveweight gain. However, the environmental data was measured within a central part of the calf building and not specifically within the pens or housing. The positioning of such recording equipment should be considered in any future work of this nature. Future work could consider night and day differences in climate related variables and the effect of this variation on the varying exposure might affect daily liveweight gain of calves. Also, further investigation should be carried out to examine the various comfort indexes and assess which is the most suitable for use with pre-weaned calves and different types of housing.

Chapter 5: The impact of the provision of bedding material and the presence of calves on the air quality within calf hutches

5.1 Introduction

Artificially reared calves are housed to protect them from extremes in climatic conditions. The housing should provide the calf with a clean, dry, draught free environment that is well ventilated. There is, however, an inevitable risk with housing that it can create a microclimate that can increase the risk of respiratory disease. The risk arises as a result of the enclosed nature of some housing systems (Roland et al, 2016). A key factor in reducing the risk of respiratory disease in such microclimates is in assessing the quality of the air. However, with respect to the livestock sector, the vast majority of research has been conducted in pig and poultry units and cattle feedlots. This is partially because the outputs of these industries are carefully monitored and margins are tight, and every incremental change which aids health and therefore growth, is actively investigated. The theme of air environment has not been extensively researched for calf rearing. There is limited published research in regards to air quality and hygiene for specific housing systems for calves such as hutches. Hill et al. (2011) identified that there was no difference in airborne bacteria levels between two different hutch types (EZ hutch™ and Calf-Tel™), which would suggest that one hutch type is not any better than the other and that management factors are the principal reasons for any differences obtained. The study by Hill et al. (2011) also suggested that airborne bacteria levels were associated with restricted airflow in hutches, and that the use of fans did not reduce airborne bacteria concentrations. There is a growing body of scientific literature examining all aspects of air quality, including particulate matter. However, this has mostly been done in horses and in pig and poultry sectors as well as cattle feedlots (Costa et al., 2009; Kaasik and Maasikmets, 2013; Papanastasiouet al., 2011; Davison et al., 2019). There is no known published scientific literature with regards to particulate matter and calf housing.

It has also been mentioned throughout this thesis that there are many different types of housing styles available but regardless of whichever type of calf housing is used there are many factors that can affect the quality of the air (Figure 5.1).

Figure 5.1 Factors affecting air quality

The use of a good ventilation system is a major preventative action that can reduce the risk of BRD in calves. The main role of ventilation is the exchange of air by the removal of products produced by the calves (heat, moisture, carbon dioxide and other gases) and airborne micro-organisms and replacing with a supply of fresh air (Wathes et al., 1983). Calf housing can be ventilated either by natural ventilation or through mechanical ventilation (e.g. positive pressure systems). Some of these factors in Figure 5.1 interact with each other, e.g. stocking density and moisture levels, temperature and health status. One example would be over-stocking a pen of calves. Overstocking runs the risk of increasing temperature levels within the pen and results in wetter bedding through additional urine which will increase the moisture levels.

Various factors highlighted in Figure 5.1 can increase the levels of particulate matter in the air. Particulate matter is a complex mix of inert items such as dead skin, hair, feed, mould spores and dust with differing biological, chemical and physical properties.

Lai et al. (2014), state that particulate matter can be classified according to the size of the particle. They can be categorised into being smaller than 10µm (referred to as PM10), smaller than 2.5 µm (referred to as PM2.5) or smaller than 1.0 µm (referred to as PM1). Martin (1967) suggested that particle size less than 5µm were particularly hazardous to calves. Webster (1984) indicates that in order to reach the lower respiratory tract, particles need to be less than 5µm in diameter as particles greater than this size are filtered from the inhaled air by the turbinate bones in the nose of the calf. Particles less than 5µm are often referred to as the respirable fraction and can end up being deposited in the alveoli of the lungs (Collins and Algers, 1986; Hinz, 2002).

Particulate matter can act as a vector for airborne micro-organisms as well as noxious gases and moisture (Zhao et al., 2014). An investigation by MacVean et al. (1986) demonstrated that particles between 2.0 and 3.3µm had an impact on the incidence of bovine respiratory disease in newly purchased cattle within feedlots. Although involving a different species (pigs), a similar conclusion was drawn by Alonso et al., (2015) where they identified porcine reproductive and respiratory syndrome virus (PPRSV) in all sizes of particles except those of 0.7 to 2.1µm in diameter, demonstrating that viruses involved in respiratory disease can be carried on particles larger than 2.1µm.

The best method to reduce particulate matter in livestock housing is to firstly prevent it from being generated (Cambra-López et al., 2011). However, this is not always possible and therefore the aim should be to keep it to a minimum. To understand the level of particulate matter that is being generated along with the airborne population of micro-organisms the levels need to be measured to allow them to be managed. Methodology for sampling air in calf housing to allow these to be managed is not well established (Hoshiba, 1986). However the possibility of sampling the quality of the air has been made more viable through the use of portable air sampling devices (Nordlund, 2008). The objective of this study was to examine the effects of two factors (presence of bedding and presence of calves) on air quality in a commercially available calf housing system (pair-rearing calf hutches), by assessing levels of particulate matter and total bacterial count. Firstly, the presence of bedding material was assessed against a completely empty hutch (no bedding material and no calves) which then led into examining the effects of calves in the hutch against no calves present. The effect of two differing qualities of bedding material was also assessed.

5.2 Materials and methods

This study was conducted at SRUC Dairy Research & Innovation Centre, Acrehead Unit, Dumfries between 14 January 2019 and 16 April 2019 (Home Office PPL P204B097E, SRUC Animal Experimental Research Committee project ED AE 35-2018).

5.2.1 Study housing

For the purpose of this study, five hutches specifically designed for paired calf rearing were used (35|85 XXL Deluxe, *Calf-Tel*, *Hammel Corporation, Wisconsin, USA*). The inside calf usable space dimensions of each hutch was 2.43m (length), 1.46m (width) and 1.36m (height). The hutches were numbered one to five and arranged side by side with a one metre space between each of them and 1.25m away from the wall of the building behind them (Figure 5.2).

Hutches one and five were on the 'exterior' of the housing set-up, and both these hutches were one metre away from metal hurdles (3m x 2.5m) that had plastic boarding attached to them. These metal hurdles acted as security fencing and as a barrier to prevent wind from entering the experimental set-up. Hutches two, three and four were regarded as being on the interior of the housing set-up. All the hutches were sited on concrete flooring. For the purpose of counting particulate matter, a circular hole, 36mm in diameter, 0.8m from the rear of the hutch and 1.1m from the base of the hutch was made in all five hutches. Only hutches that contained a pair of calves had metal hurdles attached to their front to create an 'outside' pen (2m (length), 1.46m (width-nearest hutch), 2.5m (width – away from hutch) (see Figure 5.2).

5.2.2 Study design

The aim of the study was to look at the effect of the presence of straw and straw quality as well as the effect of the presence of calves on air quality. Straw was used as the bedding material for this study with the aim of having two straw samples of contrasting quality. There were five treatments with one hutch per treatment for each replicate of the study: (i) no bedding material or calves (*No Straw – No Calves*), (ii) Straw 1 and no calves (*Straw 1 – No Calves*), (iii) Straw 1 with calves (*Straw 1 – Calves*), (iv) Straw 2 and no calves (*Straw 2 – No Calves*) and (v) Straw 2 with calves (*Straw 2 - Calves*). There were eight replicates where each replicate had three days of sample collection.

Treatment allocation (Bedding and quality (No Straw, Straw 1, Straw 2), Calves (No Calves, Calves)) for each hutch was randomised across replicate.

(i)

(ii)

Figure 5.2 Layout of study hutches (front (i) and rear (ii) view)

5.2.3 Straw bedding quality

Straw was used as the bedding material in this study as it was considered that straw is the main bedding material used for calves in the UK (Panivivat et al., 2004). Straw is regarded as a by-product from the grain industry which is not only compostable but thought to have good insulating properties. The aim was to have two straw samples of contrasting quality. A protocol for the selection of the straw was created based on visual inspection only, whereby each aspect was assigned a score (Table 5.1).

All bales were initially screened for straw colour as this was perceived as one of the main indicators of quality. Following this, signs of soil and/or other material contamination and dampness on the exterior of each bale were checked. Two bales of each quality were selected to ensure that there were sufficient stocks of each for the full duration of the study.

Table 5.1 Straw assessing methodology and assigned score

Description	Score
Exterior of bale:	
No signs of weather damage, dry to the touch, clean exterior	1
No signs of weather damage, dry to the touch but exterior contains soil marks where bale has been stored	2
Signs of weather damage, looks and feels dry to the touch	3
Signs of weather damage, looks and feels dry to the touch but vegetation growing from the exterior	4
Signs of weather damage and looks and feels wet to the touch	5
Signs of weather damage, looks and feels wet to the touch, vegetation growing from the exterior	6
Examples:   	
Straw Colour:	
Bright in appearance, light yellow/golden in colour, almost looking white	1
Starting to look dull but still looks yellow	2
Darker in colour, almost grey looking	3
Brown in appearance	4
Black in appearance	5
Examples: 1 2 3 	
Straw length:	
Short (<10cm)	3
Medium (10-20cm)	2
Long (>20cm)	1
Other vegetation:	
No signs of other vegetation present in the bale	0
Signs of other vegetation present in the bale (grass, weeds)	1

Once the bales were selected for use based on this first visual screening, they were opened. Further visual assessments using the same protocol were made on each bale such as straw length, signs of internal mould, internal presence of other vegetative material and internal soil contamination.

Five representative samples from different parts of each bale were taken and bulked into one sample per bale. A sub-sample was analysed for dry matter % (DM), ash content, g/kg DM (Ash) and modified acid determined fibre, g/kg DM (MADF).

The MADF was subsequently used to calculate the metabolisable energy content (strawME) of each straw using the following calculation:

$$\text{StrawME} = 15 - (\text{MADF} \times 0.014)$$ (McDonald et al., 2011)

The ME content is indicative of the quantity of lignin present within the straw (Webster 1984). Straw with a high ME content would indicate a straw that was less lignified and therefore less fibrous.

Table 5.2 displays the visual and analytical variation on the two straw beddings chosen and used in this study and Figure 5.3 illustrates the two straw beddings. Straw 2 was a wheat straw and was considered to be more lignified due to the lower ME content than Straw 1 which was a barley straw. From the assessment and analysis, Straw 2 was deemed of poorer quality than Straw 1.

Table 5.2 Visual and analytical assessment of the straws used in the study

	Straw 1	Straw 2
Analysis:		
DM (g/Kg)	858.0	861.5
Ash (g/KgDM)	43.8	58.6
MADF (g/KgDM)	490.5	562.5
ME	8.1	7.1
Visual assessment score:		
Exterior of bale	1	1
Straw colour	1	3
Straw length	2	1
Other vegetation	0	0
Overall visual score	4	5

DM = Dry Matter, MADF = Modified Acid Detergent Fibre, ME = Metabolisable Energy

Straw 1

Straw 2

Figure 5.3 Illustration of the two straws used as bedding material in the study

5.2.4 Straw bedding quantity

Ten kilograms of each straw bedding quality (five kilograms from each bale of that desired bedding quality) was used per hutch per day in the hutches that required straw in each replicate. This figure was selected by weighing the amount of straw that farmstaff used to bed up the individual hutches over a number of hutches (3.8kg ±0.7; mean ±sd) and then scaling up this figure accordingly for the paired–rearing hutches used in the study. Between 0815 and 0835h daily, the straw bedding material in each hutch was removed via the rear access door of the hutch. To mimic this practice, and its effect on air quality, the rear access door of the hutch containing No Straw – No Calves was opened for the length of time it would take to fully remove the straw bedding material.

5.2.5 Calves and management

Eight replicates of four calves were used within this study and encompassed a mixture of dairy (Holstein) and dairy-beef cross (Holstein - British Blue cross or Holstein – Limousin cross) calves of both sexes (male and female). The breakdown of breed and sex of the calves was as follows: Holstein: 5 female, 15 male; Holstein -British Blue cross: 3 female, 2 male; Holstein - Limousin cross: 3 female, 4 male. For the purpose of this study, each calf was seen as a 'calf unit' regardless of sex or breeds and treated generically as such. Each replicate consisted of two groups of two calves with each pairing being balanced as closely as possible for age within replicate (*Straw 1 - Calves*: 9.8d ± 2.9, *Straw 2 - Calves*: 9.9d ± 3.2 (mean ± sd) and combined liveweight (*Straw 1 - Calves*: 90.4kg ± 11.7, *Straw 2 - Calves*: 89.9kg ± 6.3 (mean ± sd). Pre-study, all calves were housed in individual Calf-Tel Compact calf hutches (*Calf-Tel, Hammel Corporation, Wisconsin, USA*) at the main calf rearing unit at SRUC Dairy Research & Innovation Centre (Crichton Royal Farm) from the point of separation from its dam. All study calves were transported to the study site between 1400 and 1500h on the day prior to the first sample collection.

Prior to transportation, the liveweight of each calf was recorded by using a calf weigh crate with *Tru-Test* digital load cells and *EziWeigh5* weigh head attached (*Ritchie Agriculture, Forfar, Angus, Scotland).* All study calves were fed reconstituted milk twice a day (0730h and 1630h) via Wydale teat feeders located in the outside pen. At each milk feed, the calves received the same volume and concentration as they previously received pre-study, which was 3 litres at each feed of 15% concentration calf milk replacer (CMR) (*OmegaGold, ForFarmers, Suffolk, England;* crude protein 23%, crude fat 18%, crude fibre 0.1%, crude ash 8.5%). Access to ad-lib water and starter pellets (VitaStart + Deccox, *ForFarmers, Suffolk, England*; crude protein 18%, crude fats & oils 4%, crude fibre 11.5%, crude ash 7%, 3mm diameter) was offered via plastic buckets on the outside pen between the two milk feeding times. Between 1630h and 0730h, the buckets containing the water and starter pellets were placed in the bucket holders within the hutch. No additional roughage apart from the bedding material was offered. Each day on the study after the 1630h milk feed, calves were visually health assessed using the Wisconsin scoring system (McGuirk, 2008). A digital thermometer (*Genia Digiflash, St. Hilaire de Chaléons, France*) was used to capture rectal temperature as part of the scoring system. Any calf that had a rectal temperature 39.5^{o}C and above and was displaying clinical signs for more than one other element of the scoring system was treated accordingly. Six calves received treatment for illness as a result of this protocol. No calf treated was removed from the hutch or the study or received treatment for more than one day. The health score given to each calf within the same hutch was then combined to give an overall ‘hutch calf health’ score.

5.2.6 Sampling process- particulate counts

Particulate counts were taken six times across a day per hutch for each of the three days for each of the eight replicates. Three samples were taken in the morning (starting at 0900h) and three taken in late afternoon (starting at 1430h). Sampling always commenced with hutch one and finished with hutch five. The first sample from each hutch was taken three minutes after bedding applied (if applicable). This sample is subsequently referred to as ‘0 mins’. The first sample was taken from hutch one each day at 0900h, and then at forty minute intervals until three samplings per hutch had been acquired. Sampling then recommenced at 1430h, again at forty minute intervals until another three samplings per hutch had been obtained (Figure 5.5). A forty minute interval between subsequent samplings per hutch was selected as this ensured that the experimenter could complete sampling for all hutches without running the risk of running into the next sampling time for the first hutch.

Particulate matter sampling was conducted using a calibrated PCE-PCO2 particle counter (*PCE Instruments UK Ltd, Southhampton, Hampshire*) and carried out by inserting the sampling head into the 36mm diameter hole in the rear of the hutch to a depth of 5cm (Figure 5.4). A sampling rate of 2.83l of air/60s was used in this study.

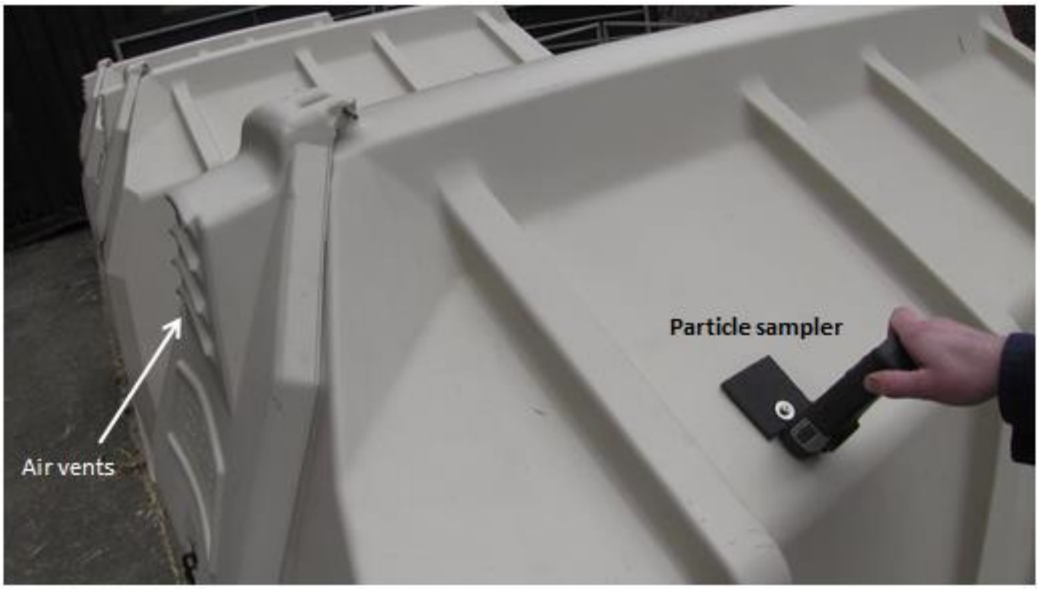
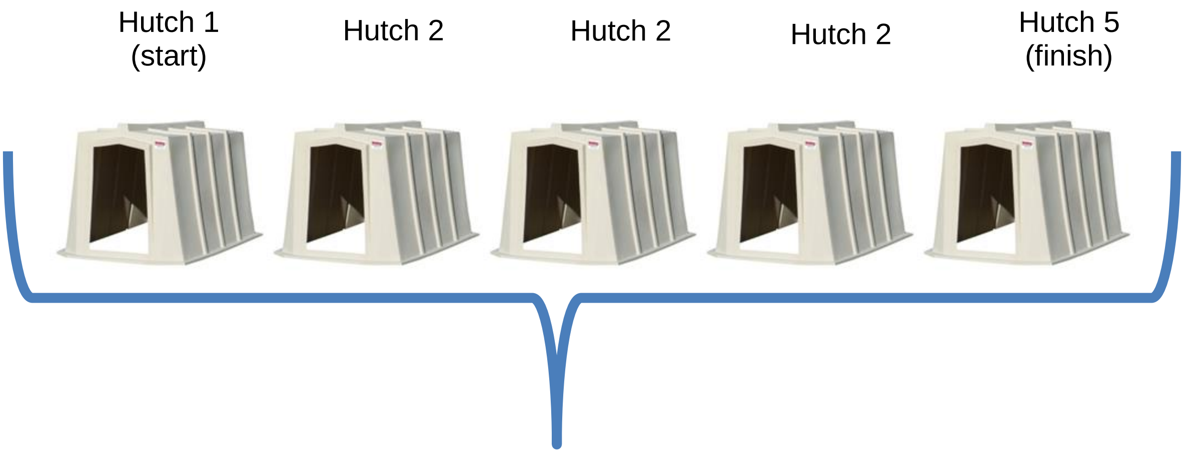


Figure 5.4 Placement of particle sampler in relation to rear air vents of hutch



Calves fed (0730h)

↓

Old bedding removed (0815-0835h)

↓

New bedding applied in hutch (as required) 0900h, Sampling 1 (0 mins after bedding applied)

↓

Sampling 2 (+ 40 mins after bedding applied)

↓

Sampling 3 (+80 mins after bedding applied)

↓

Sampling 4 (+330 mins after bedding applied) (1430h)

↓

Sampling 5 (+370 mins after bedding applied)

↓

Sampling 6 (+410 mins after bedding applied)

↓

Calves fed (1630h)

Figure 5.5 Sampling timeline

Six sizes of particulate matter were automatically recorded at every sampling – 0.3, 0.5, 1.0, 2.5, 5.0, 10.0µm respectively. For every sampling, the PCE-PCO2 created a separate file which could be downloaded and converted into a csv file for further analysis. On a small number of occasions over the course of the study, there were times when upon conversion, the files contained negative counts for all particle sizes. Such data were not included in the analysis. Also due to a technical issue with the PCE-PCO2 on day 3 of the second replicate, no particle count data was collected. Therefore, only two days of particle count data were collected for the second replicate. Another particle counter of the same make, model and specification was used for the remaining replicates of the study.

5.2.7 Sampling process – total bacterial counts

The air sample was taken using an AirIdeal-3P (*bioMérieux SA, Chemin de l'Orme,69280 Marcy-l'Étoile - France*) in partial accordance with Lago et al. (2006) which was five litres of air sampled onto 90mm diameter Columbia Blood Agar plates.

Prior to the main study initially commencing, an exercise was carried out to ensure that the whole process of obtaining the air sample for total bacterial counts was repeatable and therefore allow only one sample per sampling to be collected. Time was also taken to establish a routine that ensured that the equipment was handled in as sterile a manner as possible, given the constraints of carrying out the study in an on-farm environment. Emphasis was given to the replacement of the sampling grid during the transfer of agar plates by not touching the grid and placing the sampling grid face down during the plate transfer process as well as ensuring not to touch or breathe on the agar plate. Once satisfied that as sterile as possible technique was established, twelve samples were taken from a hutch under the same conditions and in quick succession. From this small exercise, the coefficient of variation (CV) was calculated by dividing the standard deviation by the mean number of colonies. The coefficient of variation from this exercise was found to be 20% which was deemed a suitable level of variation (Pimentel-Gomes, 2000).

On day three of each replicate, a sample of the air as well as a count of particulate matter was taken from each hutch at every sampling. This was taken immediately after the particulate sample at each recording.

Before sampling occurred on the desired day, the sampling grid of the sampler was wiped internally and then externally using an antibacterial wipe to ensure sterility and minimise contamination. Each air sample was taken at a distance of 0.45m from the interior wall of the hutch at a height of 0.9m. Any calves in close proximity of the AirIdeal -3P were at least 0.3m away from the sampling grid of the device during sampling. After the last sampling of the day, the plates were transported to the SRUC Veterinary Services Veterinary Investigation Centre, Dumfries (VIC). At the VIC, the plates were placed in a SANYO MIR-154 cooled incubator at 37^{o}C (*SANYO North America Corporation, Biomedical Solutions Division, IL 60191, USA*). After 18-24 hours (21.3hours ± 1.79, mean ± sd) the plates were removed from incubation and the number of colonies that had grown on each plate was manually counted. Training was received on colony counting from the laboratory manager at VIC.

Total bacterial counts were calculated to represent the most probable number of colonies per cubic metre (cfu/m^3) (MPNC). This was carried out by using the reading table available in the user instructions for the Air Ideal sampler. A corresponding value (most probable number of colonies, MPN) was found for the number of colonies that had been grown on each of the agar plates using Feller's law (airIDEAL, 2001). This value was then used to calculate MPNC by the following calculation which took the volume of air sampled into consideration:

MPNC (cfu/m^3) = MPN *1000/<*volume of air sampled*>

5.2.8 Calf behaviour

After each sampling, each of the calves within the hutch was recorded for place (inside hutch, outside area) and posture (standing, lying, active) (Table 5.3).

Table 5.3 Definitions of place and posture of calves

Description	Definition
Place	
Inside hutch	Calf was defined as being inside the hutch when the head and front 2 legs were within the hutch.
Outside hutch	Calf was defined as being outside the hutch when the head and front 2 legs were out-with the hutch.
Posture	
Lying	Calf was defined as lying when sternum was in contact with the ground.
Standing	Calf was defined as standing when a minimum of 3 feet were in contact with the ground.
Active	Calf was in motion: including walking (two-beat gait, with leg movements synchronized diagonally), canter (three-beat gait in between a trot and a gallop), gallop (four-beat gait with a phase where all legs were off the ground), buck (both rear legs were lifted off the ground), jump (both forelegs were lifted off the ground and body was projected upwards) and kick (one hind leg was lifted off the ground and extended to the rear or side).

5.2.9 Climate data

A Kestrel 4500 weather meter (*Nielsen-Kellerman, Birmingham, MI, USA*) was attached onto the metal hurdle adjacent to hutch 1 at a distance of 0.8m above ground level. Therefore, the climate data obtained was from outside the hutches and not within the hutches. The weather meter continuously recorded wind speed (m/s), air temperature (^oC) and relative humidity (%). A scoring system for general weather observations was created and used (Table 5.4). A reading for wind speed, air temperature and relative humidity was noted after every sampling along with general weather observations.

Table 5.4 Description and definition of general weather conditions

Description	Definition
Sunshine	Sun is visible in sky
Overcast	Sky is cloudy, dull on occasions
Precipitation	Precipitation is falling from the sky (as rain, sleet or snow)

5.2.10 Statistical analysis

All statistical analysis was conducted using R-software (R Core Team, 2016) and all data were checked for normality.

Although the particle counter assessed a wider range of particle sizes (0.3µm, 0.5µm, 1µm, 2.5µm, 5µm, 10µm), only the particle size count data from 1µm (PM_1), 2.5µm ($PM_{2.5}$), 5µm (PM_5) and 10µm (PM_{10}) were used in the analysis as these were deemed the sizes of interest based on the evidence from MacVean et al. (1986) and Alonso et al. (2015). Each particle size count was analysed individually.

To examine the effect of straw bedding in the hutch on particle count and total bacterial count, a subset of data was extracted from the overall particle count and MPNC data from the No Straw - No Calves, Straw 1 – No Calves and Straw 2 – No Calves treatments only. From this dataset, the presence of straw bedding was categorised into ‘No Straw’ and ‘Straw’ (Table 5.5). This dataset will be referred to hereafter as *STRAW*. Table 5.5 also displays the treatments involved in the comparison of straw quality (Straw 1 v Straw 2).

Another subset of data was extracted from the overall particle count and MPNC data from all the treatments except No Straw – No Calves to examine the effect of calves on particle count and total bacterial count. From this dataset, the presence of calves was categorised into 'No Calves' and 'Calves' (Table 5.5). This dataset will be referred to hereafter as *CALF*. From *CALF*, the effect of the number of calves inside the hutch (no calves, one calf, two calves) at the time of sampling on particle count per particle size and total bacterial count was also examined using the methods described in the next paragraph. Another subset of the data from *CALF* was extracted to only include data when there were one or more calves physically inside the hutch during sampling. This was to examine the effect of the posture of the calves on the count of particles per size and total bacterial count. The methods described in the next paragraph were also applied to this subset of data.

Table 5.5 Comparisons and the treatment conditions involved

Comparison: No Straw v Straw	
Term	***Treatments***
No Straw	No Straw – No Calves
Straw	Straw 1 – No Calves
	Straw 2 – No Calves
Comparison: Straw 1 v Straw 2	
Term	***Treatments***
Straw 1	Straw 1 – No Calves
	Straw 1 – Calves
Straw 2	Straw 2 – No Calves
	Straw 2 – Calves
Comparison: No Calves v Calves	
Term	***Treatments***
No Calves	Straw 1 – No Calves
	Straw 2 – No Calves
Calves	Straw 1 – Calves
	Straw 2 – Calves

For both *STRAW* and *CALF* and the subsets of *CALF*, there were five dependent variables of interest: total bacterial count (MPNC) (cfu/m^3) and four counts of particles (PM_1, $PM_{2.5}$, PM_5, PM_{10}). Using the *glmer* function of the '*lme4'* package (Bates et al., 2015), multiple univariable models were constructed to screen the independent variables for association with the dependent variables. When the dependent variable was particle count, the variables group, day, hutch and particle machine were included as random effects in the models. Only group and hutch were included in the models as random effects when the dependent variable was total bacterial count. Depending on which dataset was being analysed, the independent variables of either bedding (No Straw, Straw), or calves (No Calves, Calves) were screened along with general weather conditions (sunshine, overcast, precipitation), air temperature (^oC) (categorised by quartiles), wind speed (no wind, wind), relative humidity (%) (categorised by above and below median value), time since bedding applied (mins) (0, 40, 80, 330, 370, 410) and place of hutch (exterior (hutch 1 and 5) v interior (hutch 2, 3, and 4). For the two subsets of CALF, the independent variables were number of calves inside hutch and number of calves lying down. Variables with a p value less than 0.2 were carried forward to a multivariable linear mixed model. The multivariable generalised linear mixed model was carried out using backward stepwise selection. All particle counts and total bacterial counts were modelled using a Poisson distribution due to the right-skewed nature of the data.

5.3 Results

5.3.1 Effect of straw bedding

The results of the final models used to analyse the effect of straw bedding within the hutch on the count of particles at the four sizes are presented in Table 5.6.

When comparing the presence of straw bedding within the hutch against no straw present, there were more particles at PM_1 $PM_{2.5}$ and PM_{10} when straw bedding was present in the hutch. However for the same comparison, there were fewer particles at PM_5 when straw bedding was present in the hutch (Figure 5.6).

From the models, there was no significant difference between No Straw and Straw treatments on the count of particles at any of the four particle sizes analysed (PM_1: *p=0.461*; $PM_{2.5}$, *p=0.403*; PM_5, *p=0.466*; PM_{10}, *p=0.984*).

The results of the final model used to analyse the effect of straw bedding within the hutch on the number of colonies grown from the air sample is presented in Table 5.7.

In terms of total bacterial count, there were more colonies grown when there was straw bedding in the hutch (Figure 5.7). There was a significant effect of the presence of straw bedding on the total bacterial count based on the model used (*p=0.018*).

Table 5.6 Final model describing variables affecting particle count when comparing No Straw and Straw for the four particle sizes (PM_1, $PM_{2.5}$, PM_5,PM_{10})

	Particle count (No Straw v Straw)				
Variable	Level	Estimate	SE of estimate	P value for reference	P value for effect
PM1					
Intercept	-	1.217	0.115	-	<0.001
Straw	No	*Reference*	-	-	0.461
	Yes	0.049	0.067	0.461	
Time sample (mins)	0	Reference	-	-	<0.001
	40	-0.401	0.102	<0.001	
	80	-0.381	0.103	<0.001	
	330	-0.438	0.104	<0.001	
	370	-0.459	0.104	<0.001	
	410	-0.451	0.104	<0.001	
PM2.5					
Intercept	-	1.208	0.110		<0.001
Straw	No	*Reference*	-	-	0.403
	Yes	0.056	0.067	0.403	
Time sample (mins)	0	*Reference*	-	-	<0.001
	40	-0.420	0.103	<0.001	
	80	-0.366	0.103	<0.001	
	330	-0.420	0.104	<0.001	
	370	-0.472	0.105	<0.001	
	410	-0.420	0.103	<0.001	
PM5					
Intercept	-	0.916	0.177	-	<0.001
Straw	No	*Reference*	-	-	0.466
	Yes	-0.048	0.066	0.466	
Relative humidity (%)	<81.5	Reference	-	-	0.028
	>81.5	0.176	0.080	0.028	
PM10					
Intercept	-	1.258	0.095	-	<0.001
Straw	No	*Reference*	-	-	0.984
	Yes	-0.001	0.067	0.984	
Time sample (mins)	0	Reference	-	-	<0.001
	40	-0.459	0.104	<0.001	
	80	-0.394	0.103	<0.001	
	330	-0.440	0.104	<0.001	
	370	-0.457	0.104	<0.001	
	410	-0.440	0.103	<0.001	

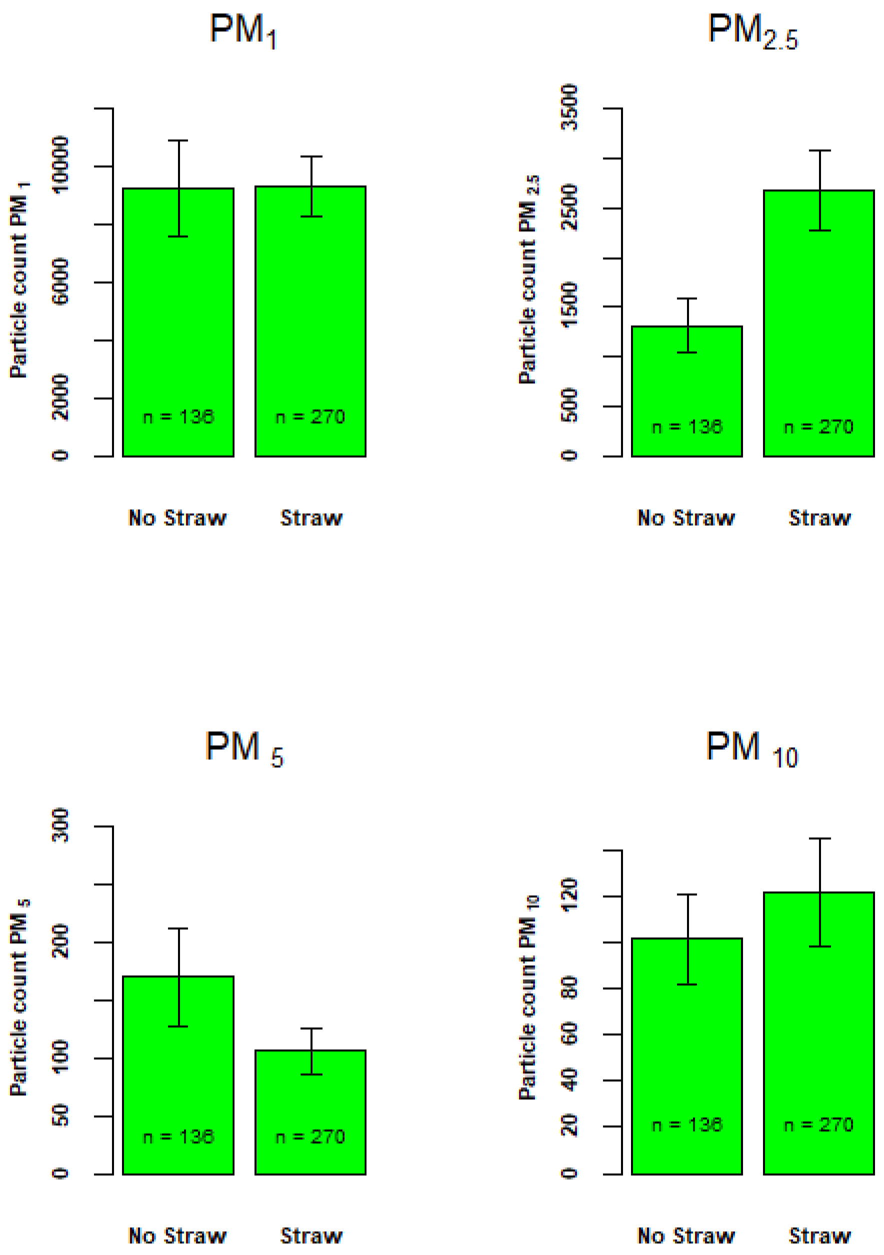


Figure 5.6 Effect of straw bedding (no straw, straw) on count of particles per particle size (n= number of observations)

Table 5.7 Final model describing variables affecting total bacterial count (cfu/m^3) when comparing No Straw and Straw

	Total bacterial count (cfu/m^3) (No Straw v Straw)				
Variable	Level	Estimate	SE of estimate	P value for reference	P value for effect
Intercept	-	0.676	0.121	-	<0.001
Straw	No	*Reference*	-	-	0.018
	Yes	0.284	0.120	0.018	

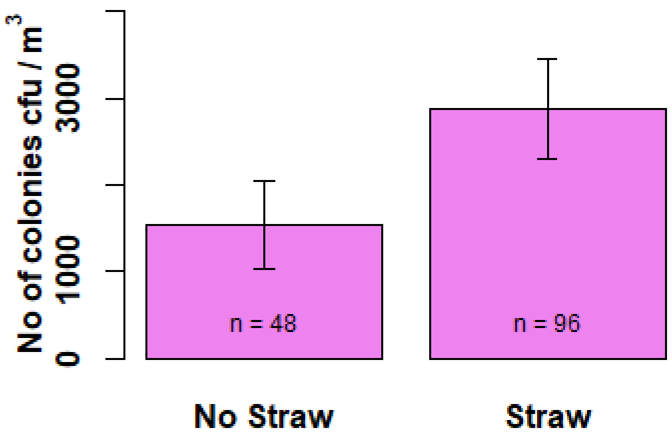


Figure 5.7 Effect of straw bedding (no straw, straw) on total bacterial count (cfu/m^3) (n= number of observations)

5.3.2 Effect of straw bedding quality

A similar count of particles was found for both of the straw qualities (Straw 1 and Straw 2), and this was consistent across the range of particle sizes (Figure 5.8).

From the models, there was no significant difference between Straw 1 and Straw 2 on the count of particles at any of the four particle sizes (PM_1, *p=0.349*; $PM_{2.5}$, *p=0.246*; PM_5, *p=0.981*; PM_{10}, *p=0.342*).

From the air samples taken from the hutches containing Straw 1, 10910.3 cfu/m^3 ± 3580.49 (mean ±se) were grown compared to 11521.8 cfu/m^3 ± 1957.43 from the hutches containing Straw 2 (Figure 5.9). There was no significant effect of straw quality (Straw 1, Straw 2) on the total bacterial count (cfu/m^3) (*p=0.360*).

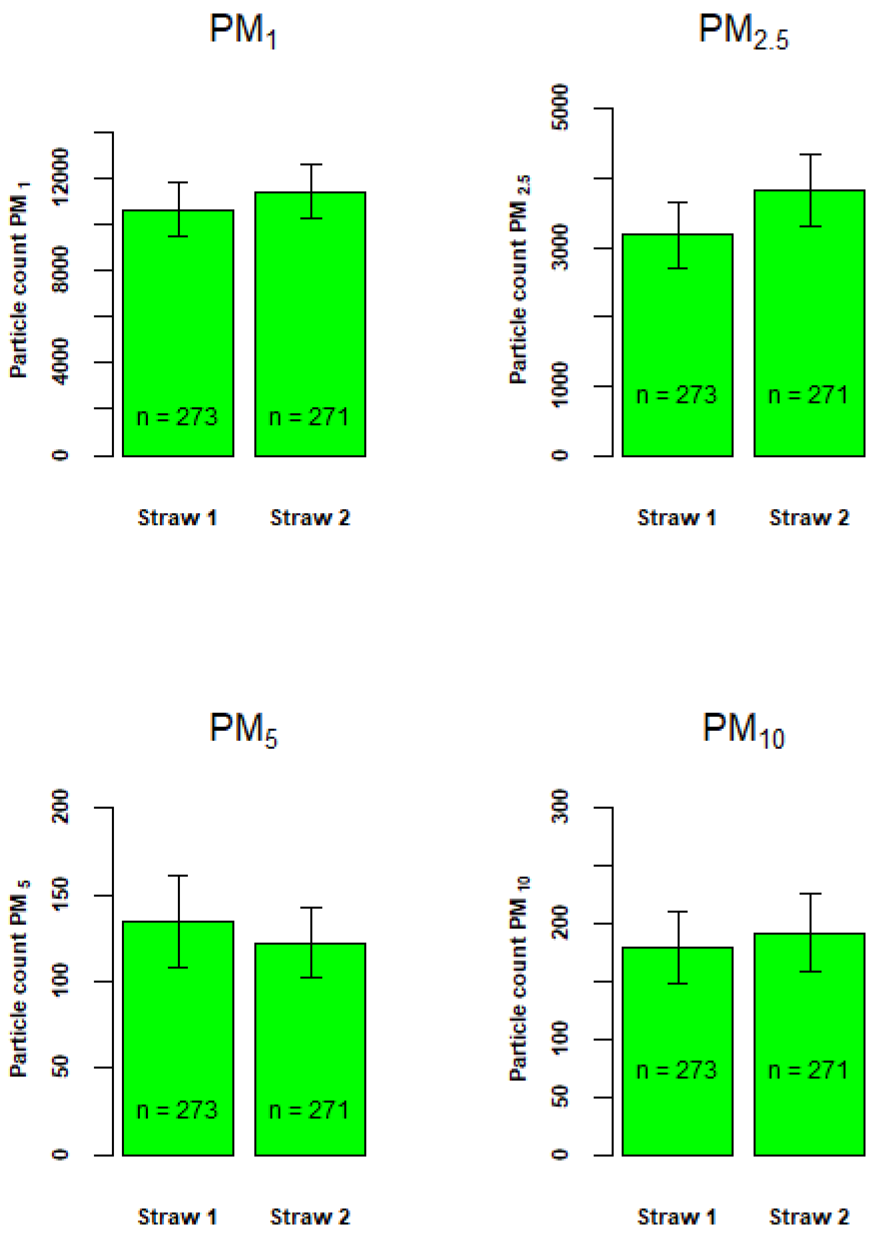


Figure 5.8 Effect of straw quality (straw 1, straw 2) on count of particles by particle size (n= number of observations)

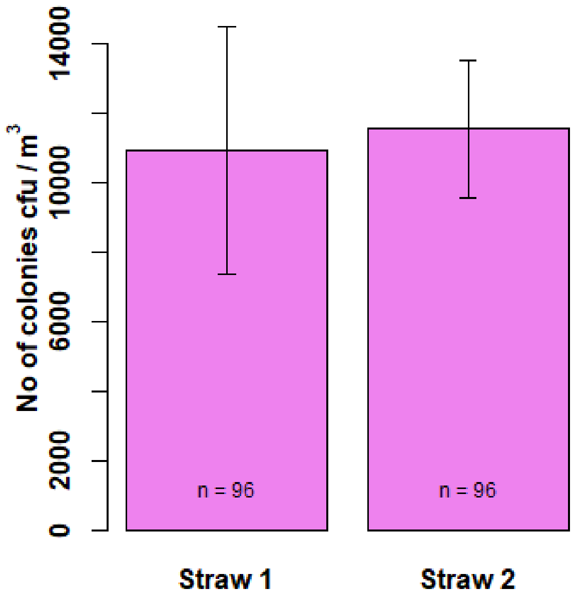


Figure 5.9 Effect of straw quality (straw 1, straw 2) on total bacterial counts (cfu/m^3) (n= number of observations)

5.3.3 Effect of calves

The results of the final models used to analyse the effect of the presence of calves on the count of particles at the four sizes are presented in Table 5.8. The presence of calves increased the count of particles in the air which was consistent across the particle sizes (Figure 5.10).

There was a significant effect of the presence of calves on the count of particles at PM_1, $PM_{2.5}$, and PM_{10} (PM_1, *p=0.041*; $PM_{2.5}$, *p=0.017*; PM_{10}, *p<0.001*). However, at PM_5, there was no significant effect of Calves on the count of particles (*p=0.095*).

Table 5.8 Final model describing variables affecting particle count when comparing No Calves and Calves for the four particle sizes (PM_1, $PM_{2.5}$, PM_5, PM_{10})

		Particle count (No Calves v Calves)			
Variable	Level	Estimate	SE of estimate	P value for reference	P value for effect
PM_1					
Intercept	-	1.294	0.092	-	<0.001
Calves	No	*Reference*	-	-	0.041
	Yes	0.111	0.054	0.041	
Time sample (mins)	0	*Reference*	-	-	<0.001
	40	-0.591	0.089	<0.001	
	80	-0.543	0.089	<0.001	
	330	-0.542	0.088	<0.001	
	370	-0.556	0.089	<0.001	
	410	-0.532	0.088	<0.001	
$PM_{2.5}$					
Intercept	-	1.274	0.080	-	<0.001
Calves	No	*Reference*	-	-	0.001
	Yes	0.175	0.054	0.001	
Time sample (mins)	0	*Reference*	-	-	<0.001
	40	-0.634	0.090	<0.001	
	80	-0.574	0.089	<0.001	
	330	-0.554	0.088	<0.001	
	370	-0.583	0.089	<0.001	
	410	-0.507	0.087	<0.001	
PM_5					
Intercept	-	0.951	0.187	-	<0.001
Calves	No	*Reference*	-	-	0.095
	Yes	0.091	0.054	0.095	
PM_{10}					
Intercept	-	1.092	0.067	-	<0.001
Calves	No	*Reference*	-	-	
	Yes	0.401	0.055	<0.001	
Time sample (mins)	0	*Reference*	-	-	<0.001
	40	-0.587	0.091	<0.001	
	80	-0.575	0.092	<0.001	
	330	-0.432	0.087	<0.001	
	370	-0.502	0.089	<0.001	
	410	-0.414	0.087	<0.001	

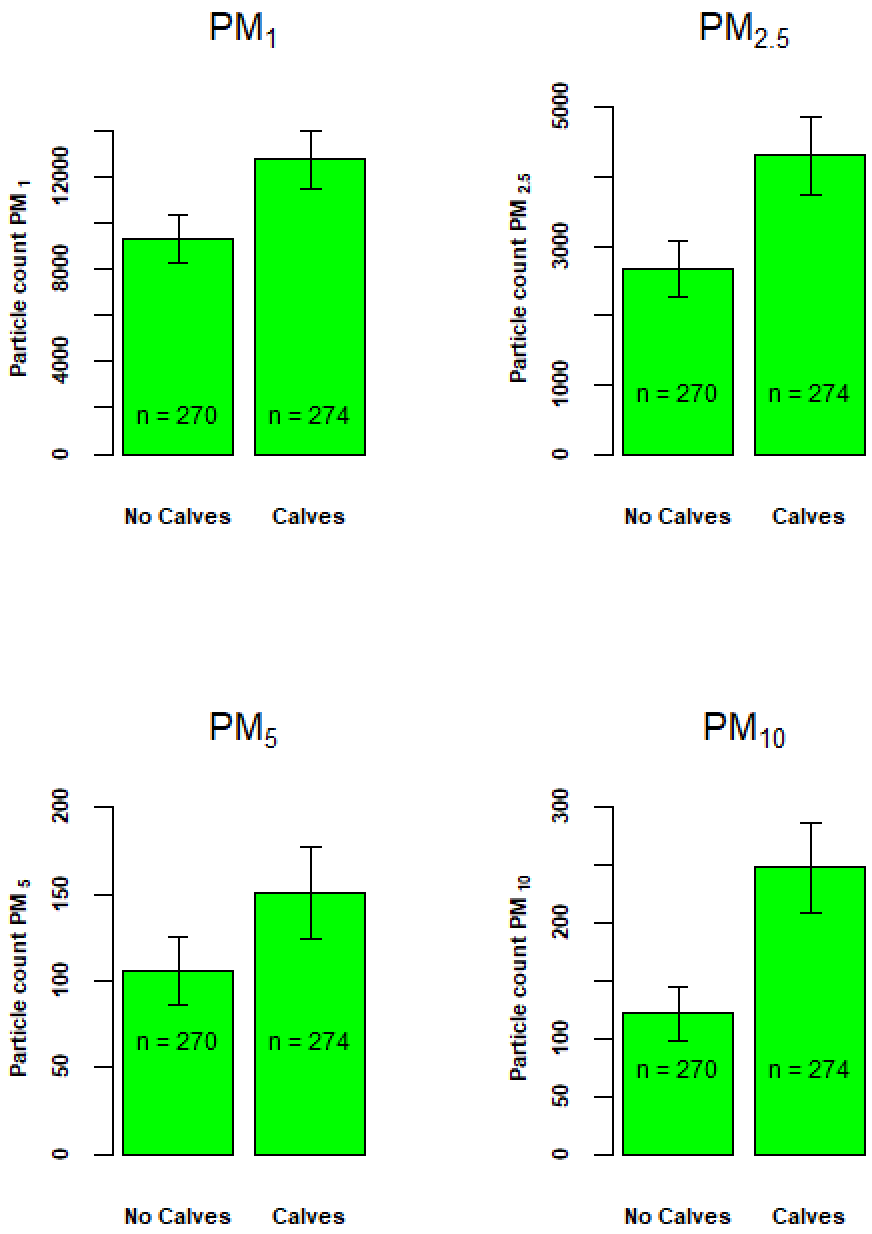


Figure 5.10 Effect of calves (no calves, calves) on count of particles by particle size (n= number of observations)

The result of the final model used to analyse the effect of the presence of calves within the hutch on the number of colonies grown from the air sample is presented in Table 5.9.

Table 5.9 Final model describing variables affecting total bacterial count (cfu/m^3) when comparing No Calves and Calves

	Total bacterial count (cfu/m^3) (No Calves v Calves)				
Variable	Level	Estimate	SE of estimate	P value for reference	P value for effect
Intercept	-	2.034	0.075	-	<0.001
Calves	No	*Reference*	-	-	<0.001
	Yes	0.277	0.064	<0.001	
Time sample (mins)	0	*Reference*	-	-	0.033
	40	-0.289	0.107	0.007	
	80	-0.309	0.108	0.004	
	330	-0.238	0.106	0.024	
	370	-0.226	0.105	0.032	
	410	-0.131	0.103	0.201	

There were considerably more colonies grown from the air samples taken from hutches that had calves than those that did not have calves (No Calves: 2878.1cfu/m^3 ± 575.45; Calves: 19706.6cfu/m^3 ± 6951.40 (mean ±se) (Figure 5.11). There was also a significant effect of the presence of calves on the total bacterial count (cfu/m^3) (*p<0.001*).

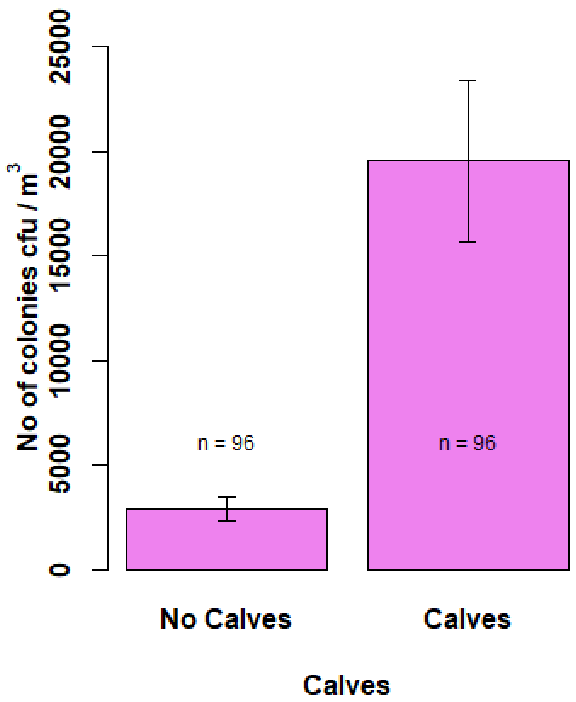


Figure 5.11 Effect of calves (no calves, calves) on total bacterial count (cfu/m^3) (n= number of observations)

5.3.3.1 Number of calves

For all four sizes of particles, the count of particles was lower when there were two calves in the hutch at the time of sampling compared to when there was no calves of one calf in the hutch (Figure 5.12). This trend was also seen for the total bacterial count (cfu/m^3) (Figure 5.13). There was no significant effect of the number of calves in the hutch at the time of sampling on the count of particles for PM_1 and PM_5 (PM_1, *p=0.140*; PM_5, *p=0.588*) but there was a significant effect for $PM_{2.5}$ and PM_{10} ($PM_{2.5}$, *p=0.026*; PM_{10}, *p=0.017*). Following post-hoc tests, there were no significant differences found for either $PM_{2.5}$ or PM_{10}. There was also no significant effect of number of calves on MPNC (*p=0.064*).

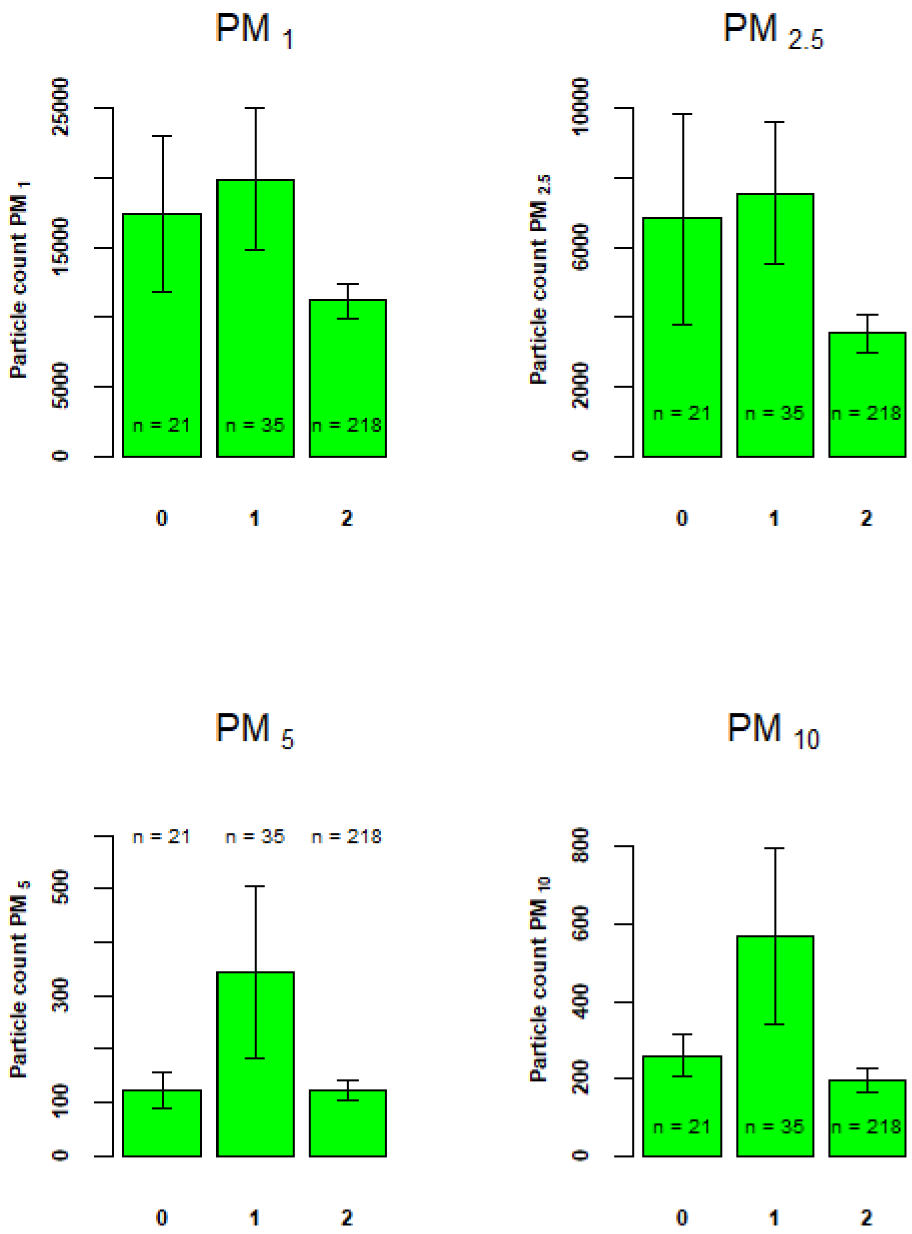


Figure 5.12 Effect of the number of calves (0, 1, 2) on count of particles by particle size (n= number of observations)

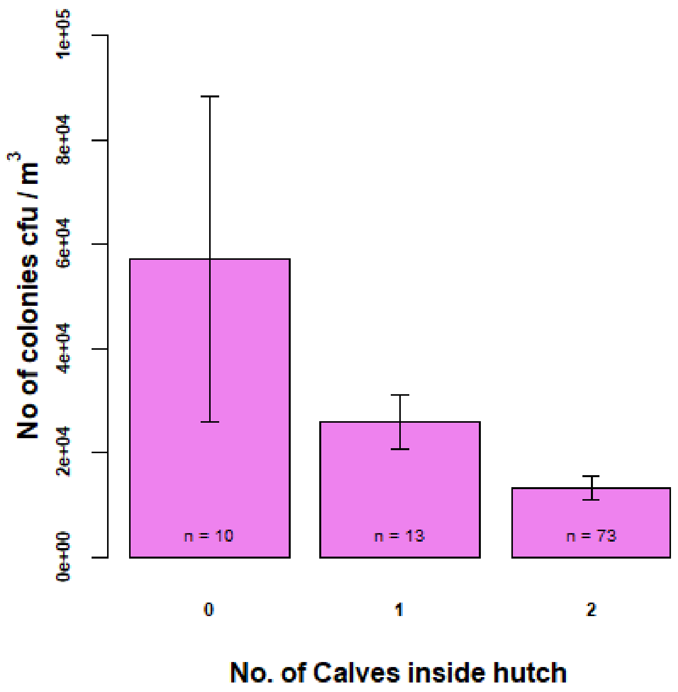


Figure 5.13 Effect of the number of calves (0, 1, 2) on the total bacterial count (cfu/m^3) (n= number of observations)

5.3.3.2 Calf posture

Calves were seen lying down inside the hutch on 72% of the observations across the study (Table 5.10). There were a higher proportion of the observations when the calves were standing after some time had passed since the straw bedding was applied in the hutch.

Table 5.10 Proportion of observations calves were observed lying, standing or active within the hutch

Calf	Time after straw bedding applied (mins)						
Posture	0	40	80	330	370	410	Study
Lying	0.55	0.98	0.93	0.60	0.61	0.63	0.72
Active	0.09	0.00	0.00	0.22	0.09	0.14	0.09
Standing	0.36	0.02	0.07	0.18	0.30	0.23	0.19

For all sizes of particles there were fewer particles when two calves were observed lying down compared to when no calves were lying down (Figure 5.14).

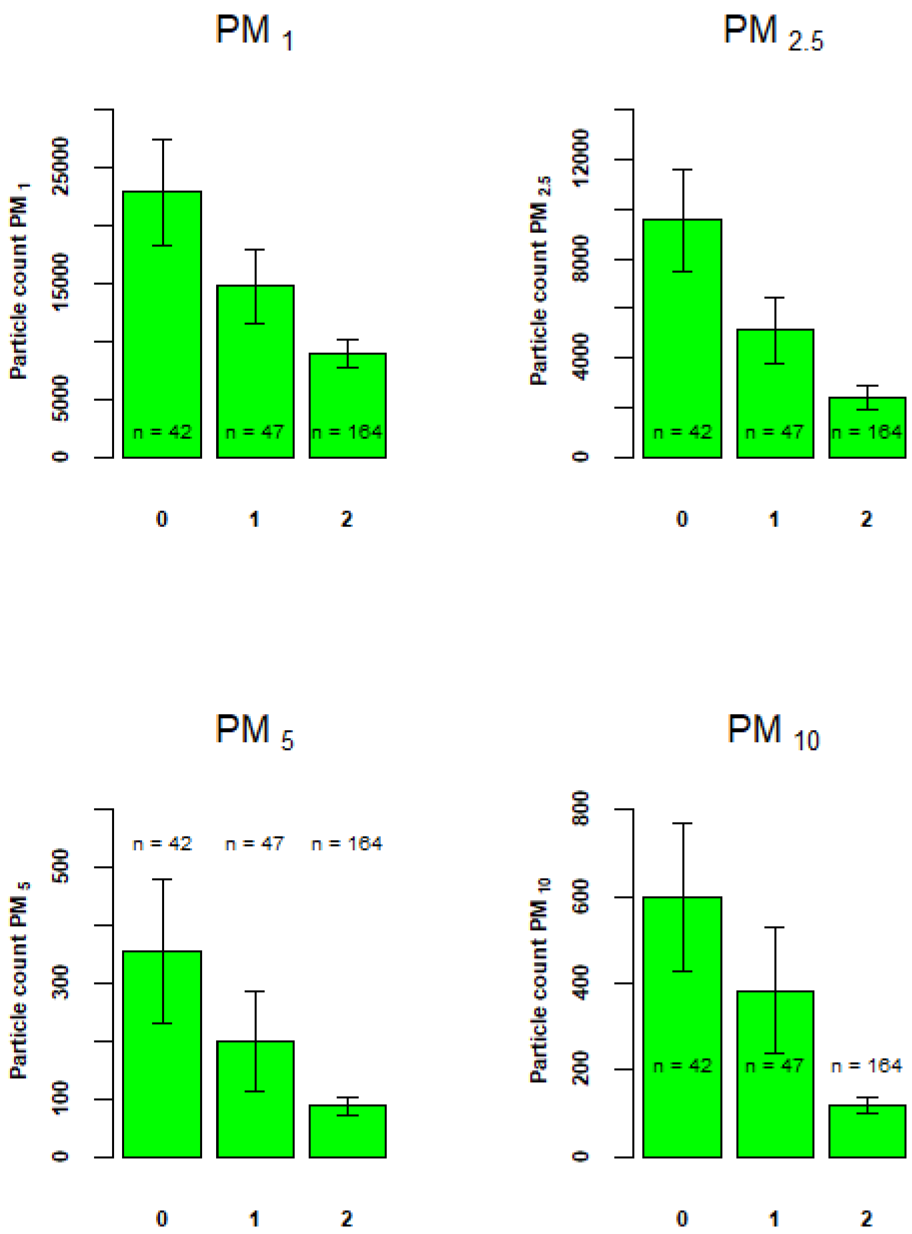


Figure 5.14 Effect of the number of calves observed lying down (0, 1, 2) on the count of particles by particle size (n= number of observations)

From the model based on data when calves were only observed as being inside the hutch at the time of sampling, there was a significant effect of the number of calves observed lying down on the particle count for PM_1, $PM_{2.5}$ and PM_{10} (PM_1, *p=0.001*; $PM_{2.5}$, *p<0.001*; PM_{10}, *p<0.001*). There was no significant effect for PM_5 (*p=0.119*). Following post-hoc tests, there were significant differences seen between no calves (0) and two calves lying down on the particle count for PM_1, $PM_{2.5}$ and PM_{10} (PM_1, *p=0.002*; $PM_{2.5}$, *p<0.001*; PM_{10}, *p<0.001*). There were fewer particles when there was two calves observed lying down in the hutch compared to when there was no calves lying down. For $PM_{2.5}$ and PM_{10} only, there was also a significant difference seen between one and two calves lying down ($PM_{2.5}$, p=0.034; PM_{10}, *p<0.001*).

Fewer colonies were grown when there were two calves lying down inside the hutch compared to when there was either no calves or one calf lying down (Figure 5.15). A significant effect was seen for the number of calves lying down on the total bacterial count (cfu/m^3) (*p=0.007*). Following post-hoc tests, there was a significant difference found between no calves (0) and two calves (*p=0.009*).

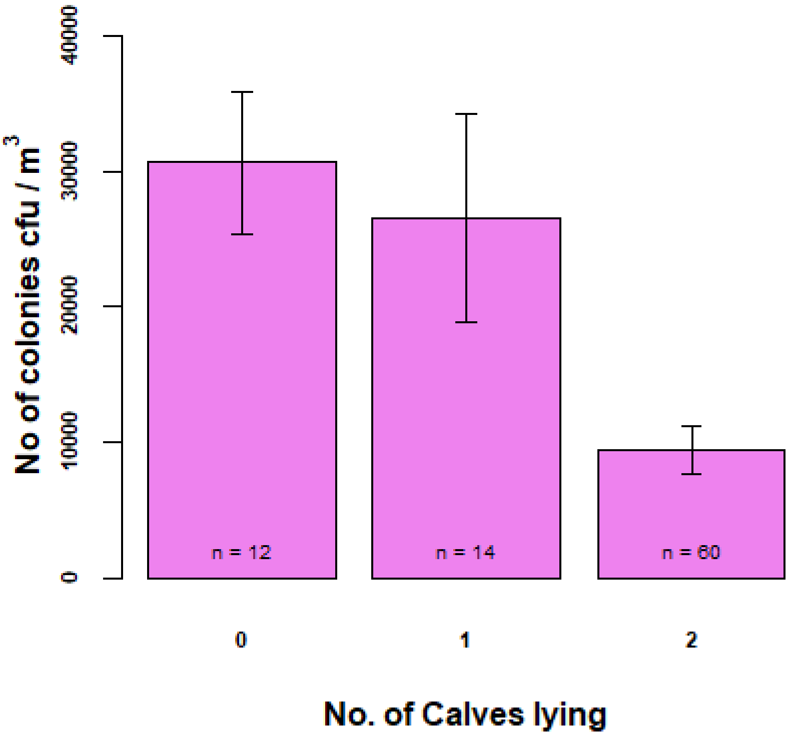


Figure 5.15 Effect of the number of calves observed lying down (0, 1, 2) on total bacterial count (cfu/m^3) (n= number of observations)

5.3.4 Time after straw bedding applied

Particle counts for all four particle sizes (PM_1, $PM_{2.5}$, PM_5, PM_{10}) were lower at 40, 80, 330, 370 and 410mins compared to 0mins after straw bedding was applied when examining the effects of the presence of straw bedding (Straw v No Straw) (Figure 5.16). There was a significant effect of time after bedding being applied on the count of particles at PM_1 ($p<0.001$), $PM_{2.5}$ ($p<0.001$) and PM_{10} ($p<0.001$). There was no significant effect at PM_5 ($p=0.345$). Following post hoc tests, for PM_1 there was a significant difference between 0 and 40mins after bedding being applied on particle count ($p=0.001$), 0 and 80mins ($p=0.003$), 0 and 330mins ($p<0.001$), 0 and 370mins ($p<0.001$) and between 0 and 410mins ($p<0.001$). For $PM_{2.5}$, significant differences were found between 0 and 40mins ($p<0.001$), 0 and 80mins ($p=0.005$), 0 and 330mins ($p<0.001$), 0 and 370mins ($p<0.001$) and between 0 and 410mins ($p<0.001$). Significant differences were also found for PM_{10} between 0 and 40mins after bedding was applied (*p<0.001*), 0 and 80mins (*p=0.002*), 0 and 330mins *(p<0.001*), 0 and 370mins (*p<0.001*) and between 0 and 410mins (*p<0.001*).

When examining the effect of the presence of straw bedding, there were fewer colonies grown from the air sample taken 40mins after the straw bedding being applied and subsequent times compared to 0mins (Figure 5.17). There was no significant effect of the time after the bedding was applied on MPNC (*p=0.069*).

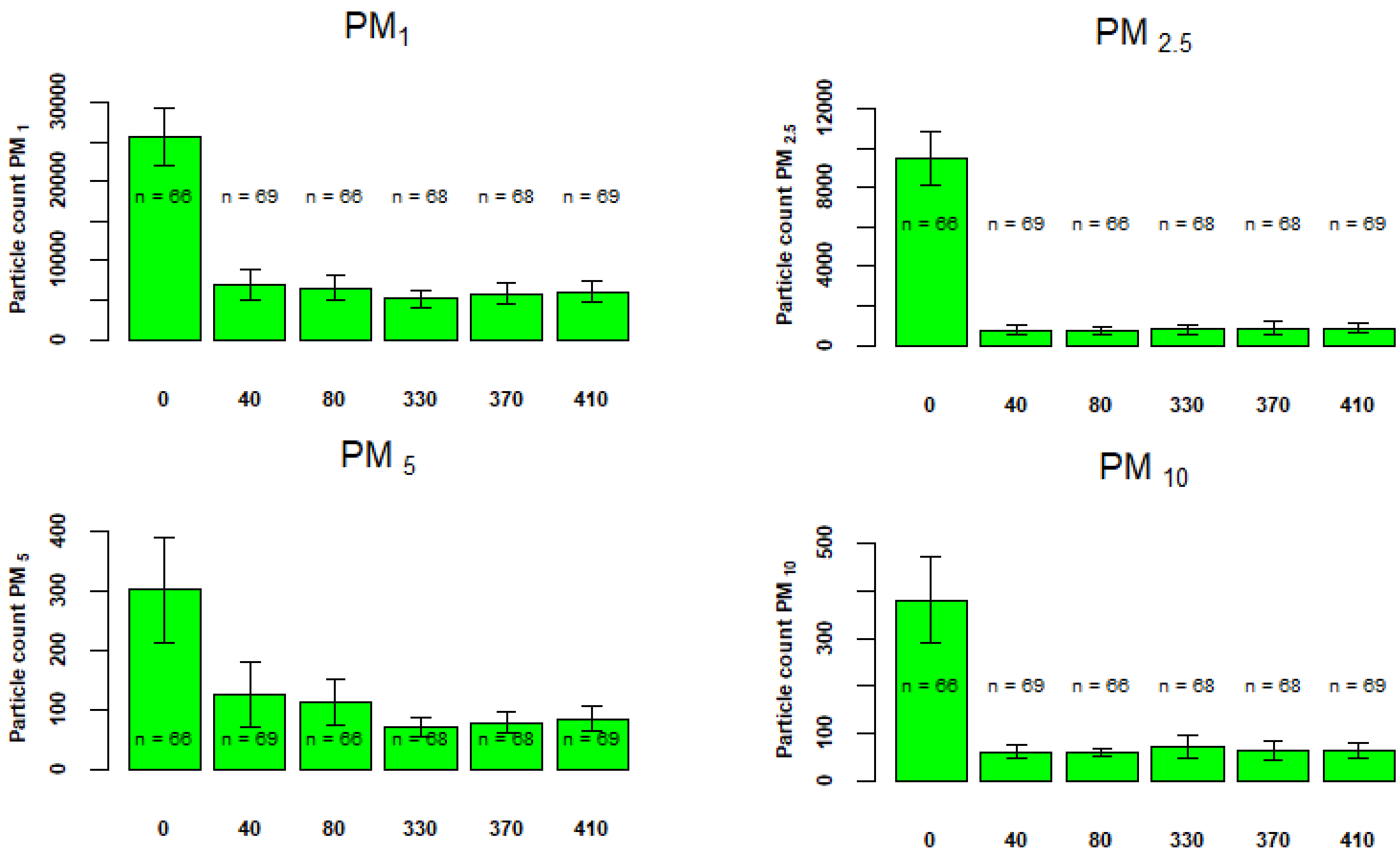


Figure 5.16 Effect of time after bedding applied (0,40,80,330,370,410mins) on particle count by particle size when examining the presence of straw (n= number of observations)

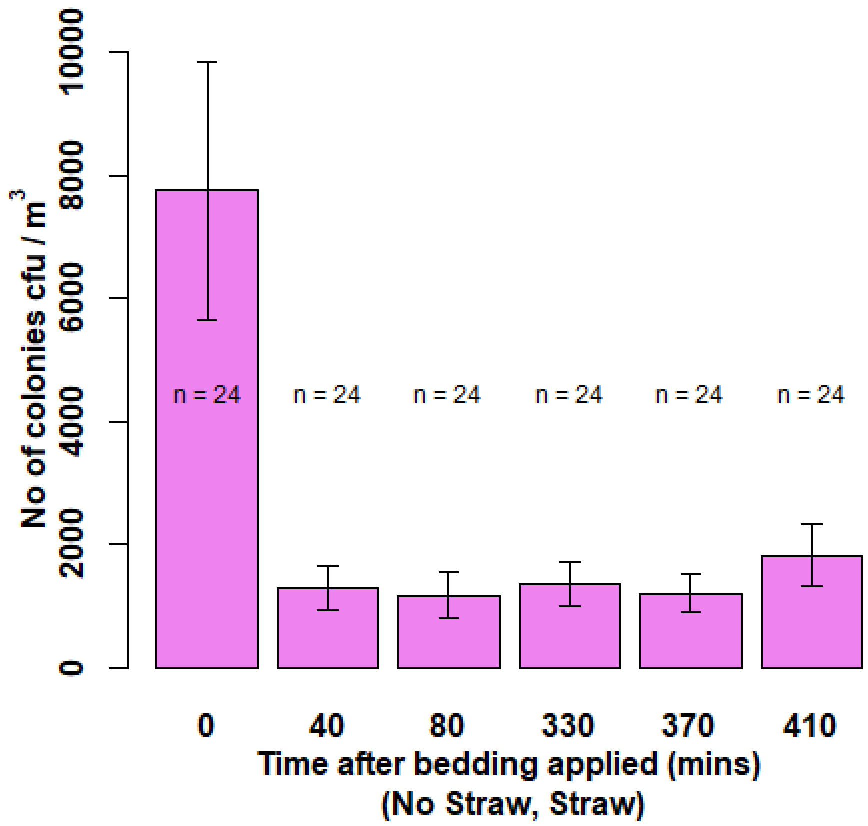


Figure 5.17 Effect of time after bedding applied (0,40,80,330,370,410mins) on total bacterial count (cfu/m^3) when examining the presence of straw (n= number of observations)

Particle counts for all four particle sizes (PM_1, $PM_{2.5}$, PM_5, PM_{10}) were lower 40, 80, 330, 370 and 410mins after straw bedding was applied when examining the presence of calves (No Calves v Calves) compared to 0mins (Figure 5.18). There was a significant effect of time after bedding being applied on the count of particles at PM_1 (*p<0.001*), $PM_{2.5}$ (*p<0.001*) and PM_{10} (*p<0.001*). There was no significant effect at PM_5 (*p=0.927*). Following post hoc tests, for PM_1 there was a significant difference between 0 and 40mins after bedding being applied on particle count (*p<0.001*), 0 and 80mins (*p<0.001*), 0 and 330mins (*p<0.001*), 0 and 370mins (*p<0.001*) and between 0 and 410mins (*p<0.001*). For $PM_{2.5}$, significant differences were found between 0 and 40mins (*$p<0.001$*), 0 and 80mins (*$p<0.001$*), 0 and 330mins (*$p<0.001$*), 0 and 370mins (*$p<0.001$*) and between 0 and 410mins (*$p<0.001$*).

Significant differences were also found for PM_{10} between 0 and 40mins after bedding was applied (*$p<0.001$*), 0 and 80mins (*$p<0.001$*), 0 and 330mins (*$p<0.001$*), 0 and 370mins (*$p<0.001$*) and between 0 and 410mins (*$p<0.001$*).

When examining the presence of calves, there were fewer colonies grown from the air sample taken 40mins after the straw bedding being applied compared to 0mins (Figure 5.19). After 40mins, the total bacterial count (cfu/m^3) increased. There was a significant effect of the time after straw bedding was applied on MPNC (*$p=0.012$*). There was a significant difference found between 0 and 40mins after straw bedding was applied on MPNC (*$p=0.030$*) and between 0 and 80mins (*$p=0.018$*).

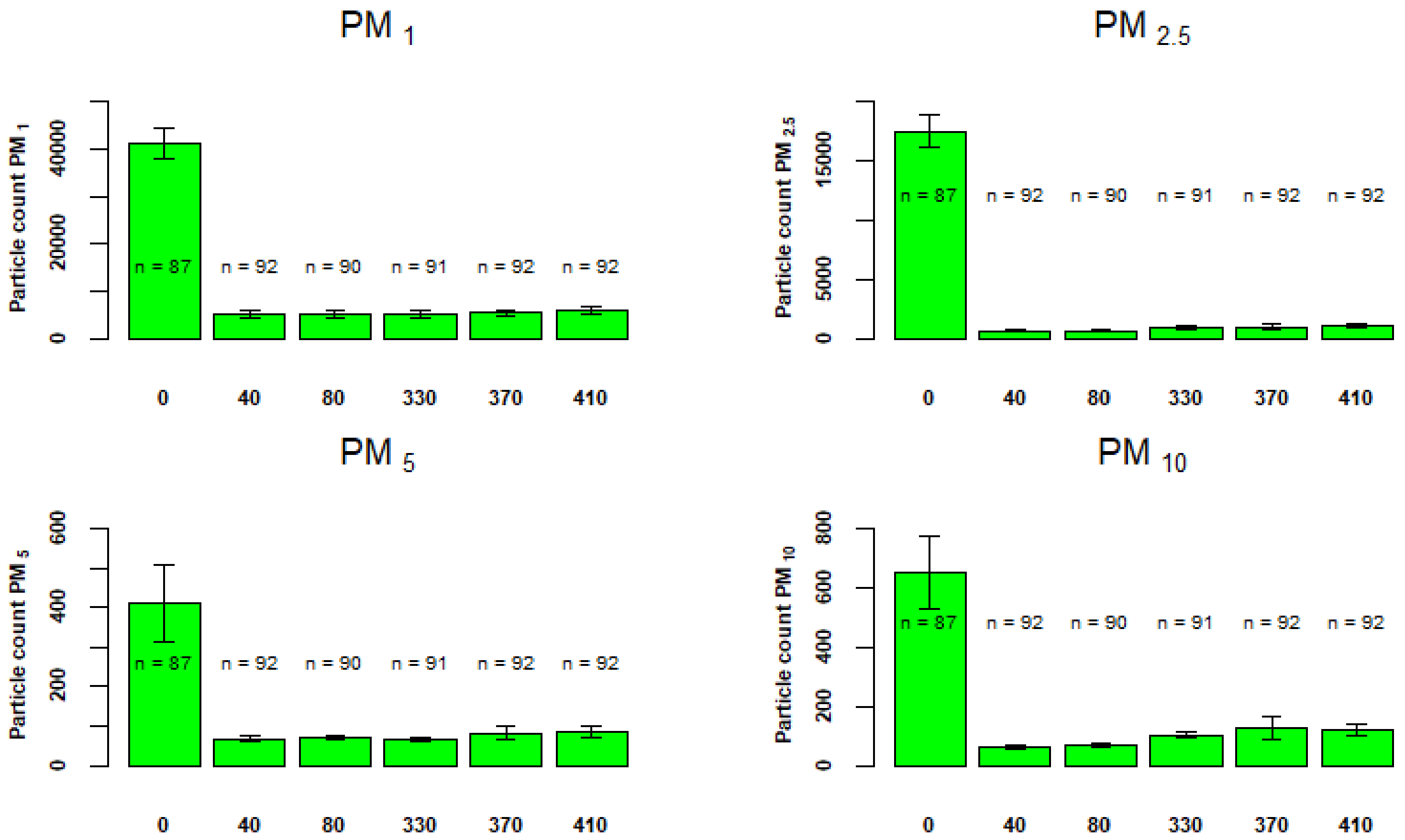


Figure 5.18 Effect of time after bedding applied (0,40,80,330,370,410mins) on particle count by particle size when examining the presence of calves (n= number of observations)

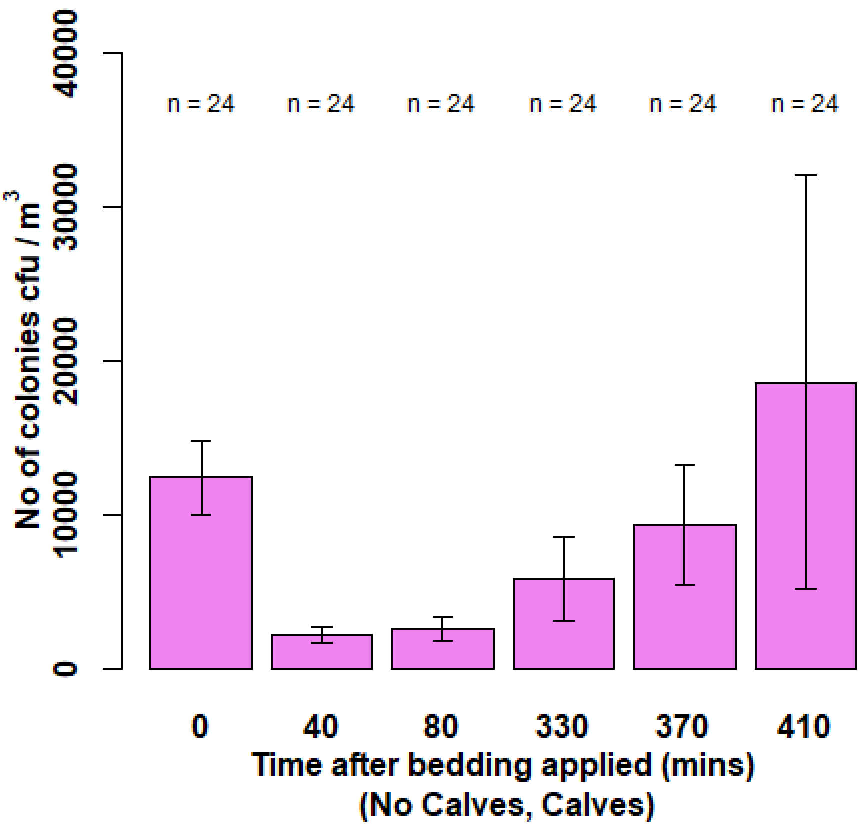


Figure 5.19 Effect of time after bedding applied (0,40,80,330,370,410mins) on total bacterial count (cfu/m^3) when examining the presence of calves (n= number of observations)

5.3.5 Weather conditions and climate

In terms of general weather conditions, 63.6% of the particle counts were taken when it was overcast, 27.7% in sunshine and 8.7% when it was raining. When samples of the air were taken for total bacterial counts, 68.3% were taken in overcast conditions and the remaining 31.7% were taken in sunshine. Unintentionally, no samples for total bacterial counts were taken when it was classified as precipitation. Table 5.11 describes the climatic conditions under which particle counts and samples for total bacterial counts were taken. The fourth sampling of particulate (330mins after bedding applied) was taken at the highest temperature across the sampling day

(Appendix A, Table A-1). This trend also occurred with total bacterial counts (Appendix A, Table A-2). General weather conditions did not have any significant effect on particle count at any of the four particle sizes ($p>0.05$) or MPNC ($p>0.05$) when examining the presence of straw (No Straw v Straw) or the presence of calves (No Calves v Calves). Based on the results of the models, wind speed, air temperature and relative humidity had no significant effect on particle count at PM_1, $PM_{2.5}$ and PM_{10} ($p>0.05$) or MPNC ($p>0.05$) when examining the effects of the presence of straw within the hutch. For PM_5, there was no significant effect of wind speed or air temperature but there was a significant effect of relative humidity ($p=0.028$) when examining the presence of straw (No Straw v Straw).

Table 5.11 Description of climatic conditions under which sampling occurred

	Mean	Median	Range
Particle count:			
Wind speed (m/s)	0.3	0.0	0.0 , 1.9
Temperature (^{o}C)	8.8	9.6	-4.2 , 19.5
Relative humidity (%)	81.5	82.1	32.9 , 100.0
Total bacterial count:			
Wind speed (m/s)	0.3	0.0	0.0 , 1.5
Temperature (^{o}C)	8.1	7.8	-1.1 , 19.5
Relative humidity (%)	82.3	85.4	41.2 , 100.0

5.4 Discussion

5.4.1 No Straw – No Calves treatment

The No Straw – No Calves treatment was essentially an empty hutch and represented the background environment and was used as the 'control' to assess the effect of straw bedding on air quality. The introduction of straw as a bedding material for the hutch created a two-fold increase in the number of particles at $PM_{2.5}$ and increased the number of particles at PM_1 and PM_{10}. The presence of calves in addition to straw bedding increased the number of particles yet further. In the case of PM_{10} there were 2.4 times more particles compared to the empty hutch (No Straw, No Calves) and approximately a 3.3 times increase in $PM_{2.5}$ for the same comparison. Therefore it can be concluded that the presence of the bedding material and calves were contributing to the measured particulate readings. This result concerning the control treatment is replicated by Nazarenko et al. (2018). Their study involving horses which were stalled found that the measurements with no animals and no bedding were considerably lower than when animals and bedding was present.

Particle size 5µm (PM_5) produced some unexpected particle counts for the various treatments. For this particle size, the particle counts for No Straw – No Calves were higher than when the straw bedding and the straw bedding with calves present. From this result, it could be assumed that there were more particles of this size circulating in the background environment than were produced from the straw bedding. The fact that there was a higher count of particles of this size when calves were present would indicate that the calves were producing some particles that were 5µm. However, this still does not explain why the count at PM_5 when straw and calves were present was less than for the empty hutch.

5.4.2 Effect of straw bedding and straw bedding quality

Straw was used as the bedding material in this study as it was considered that straw is the main bedding material used for calves in the UK (Panivivat et al., 2004). It could be considered that the two straw bedding types used for this study were not sufficiently different in quality to demonstrate a difference in particulate levels. Several other studies (Curtis et al., 1996; Fleming et al., 2008; Kwiatkowska-Stenzel et al., 2017; Nazarenko et al., 2018) have examined the effect of various types of bedding material ranging from straw to paper and wood shavings on particulate matter. The consensus from these studies was that straw generated the highest concentration of particulate matter. Lago et al. (2006) established that higher bacterial counts were found in calf pens where the bedding material was straw rather than wood shavings or sawdust. Despite this, the authors of that study believed that the thermal benefits of the straw bedding out-weighed the associated higher bacterial counts.

In this study, the straw bedding was removed daily from each hutch which would not reflect normal farm practice which would be to 'top-up' the bedding and remove it when the calf was removed. The removal of the straw bedding each day was necessary for this study so that each day was repeatable and data could be compared across days and replicates under the same hutch management conditions. It could be assumed that under a normal farm practice there could possibly be more particles in the air due to the accumulation of manure, flakes of skin and hair within the hutch.

5.4.3 Effect of calves

This study found that the presence of calves had a significant effect on the count of particulate matter and the number of colonies grown from an air sample. Banhazi (2011) reported that the number of animals (in a study using pigs) had a significant association with the concentration of particles. This result follows a similar trend found by Nazarenko et al. (2018) who found from their study involving horses in stalls that the horses were an important driver of the release and resuspension of particulate matter due to their activity. Wathes et al. (1984) reported that calves in their study were a major source of bacteria and they also found that when the calves were removed at the end of the trial, levels fell to less than one-sixth of those with calf occupancy.

It could be considered that the presence of calves is providing another potential source of particulate matter into the hutch environment through the shedding of hair, skin etc. This study has shown that there was a significant difference in particle count between hutches with and without calves. There were more particles when calves were present. It also showed that when the maximum numbers of possible calves (two) were inside the hutch the count of particles was lower than when there was only one calf or no calves inside the hutch. This result is in reverse of that stated in the review by Tan and Zhang (2004) who say that as stocking density increases then it is likely that the concentration of particulate matter will increase. The calves in this study were observed lying down 68% of the time. Therefore, it could be suggested that the calves were suppressing the suspension of particulate matter and bacteria into the air. Overall, it has been shown that the presence of calves increases particulate matter as the calves themselves were a source of particles. Another possibility is that any activity by the calves has the potential to disturb the straw and any particulate matter that had settled and distribute it into the air.

The air samples grown from the hutches containing calves had a mean value of 19706.6cfu/m^3 (19.706 x 10^{3}cfu/m^3). This value is relatively low in comparison to those found by Lago et al. (2006) where they recorded a mean value of 110631cfu/m^3 (110.6 x10^{3}cfu/m^3) within the calf pens and a minimum value of 5002 cfu/m^3 (5.002 x10^{3}cfu/m^3). The mean value obtained in this study is considerably lower than that found by Lee et al. (2005) in clean pig housing (140 x10^{3}cfu/m^3) and by Bródka et al. (2012) within intensive poultry production with a litter bedding system (5.69 x10^{7}cfu/m^3). The slightly low value obtained in this study may possibly be due to the design of the study in that the bedding was removed every day and therefore bacteria was not given the chance to multiply. As far as the author is aware, there are no known acceptable total airborne bacterial levels for calves.

5.4.4 Time

From the results of this study, it can be seen that there is a large presence of particles in the air at the point of bedding (0mins) that decreases nearly nine fold by the time of the second sampling, which was forty minutes post-bedding application. Webster et al. (1987) found in their study that the process of bedding down using straw increased the concentration of particles greater than 0.5µm by six times. Therefore the results of both studies would indicate that the process of bedding down introduces an ‘at risk’ period for additional particles to be added to the air which can potentially carry micro-organisms which the calf could breathe in.

With regards to total bacterial count, after straw bedding was applied there was a sharp decrease and then a gradual increase in the number of colonies over time. A possible theory for this could be that some of the bacteria obtained were thermophilic, and therefore as the air temperature across the day increased, ideal conditions were created to allow such bacteria to proliferate. Such a theory is mentioned by Lago et al. (2006) who stated that the increase in temperature within the calf pen was related to an increase in the total bacterial count. Another possible explanation could be concerning the posture of the calf at sampling. Examining the proportion of observations where the calf was standing (Table 5.10) along with Figure 5.18 it can be seen that they both follow a similar trend where after the initial sampling there is a decrease in time spent standing to the next sampling time and then a gradual increase over the subsequent samplings. Therefore it could be assumed that there is an association between total bacterial count and the posture of the calf in that bacterial counts were higher when calves were standing. As previously mentioned, during sampling the device was at least 0.3m away from the calves.

5.4.5 Climate and weather conditions

The climatic parameters of air temperature, relative humidity and wind speed had no significant effect on particle count at PM_1, $PM_{2.5}$ and PM_{10} or MPNC in this study. This result follows that found by Fleming et al. (2008). In their study examining the generation of particles from different bedding materials that were used in horse stables, they found that the air temperature and relative humidity were not significant sources of variation for PM10 concentration. However, in the present study, relative humidity was found to have a significant effect on the count of particles at PM_5 when examining the effects of the presence of straw (No Straw v Straw). Lai et al. (2014) showed that relative humidity had no significant effect on particle count although there was a tendency for the count for $PM_{2.5\text{-}10}$ to be lower at higher humidity levels. No explanation can be given as to why relative humidity levels affected the count for PM_5 and none of the other particulate levels analysed when examining the presence of straw.

5.4.6 Calf health

The results obtained in this study indicate a number of issues in relation to calf health. First and foremost, the calves themselves are a source of particulate matter and bacteria generation that can increase the particle and bacterial load in the environment for the calf and its respiratory tract. The calves also act as a means of agitating and disturbing the bedding material which will cause the release of particles and bacteria. MacVean et al. (1986) mentioned that particles between 2.0 and 3.2µm had a significant effect on the incidence of BRD. The results suggest (see Figure 5.10) that the calves in this study increased the count of particles at 2.5µm and a cautious approach towards the number of calves housed together should be taken. The process of bedding down also introduces another addition to the particle and bacterial load which adds potential risk to the health of the calf. Although not practically possible in many housing systems, to reduce particle and bacterial load on the calf, a solution to this potential risk would be to remove the calf from the situation when bedding down is being carried out. Particle count also drops very quickly after bedding up, so the calf would not need to be removed for very long. However, moving calves to bed up can cause stress and potentially increase the risk of disease to the calf. Therefore, there would have to be a balance as to which practice was more of a risk to the calf – keep the calf within the housing during bedding or moving to another area.

5.4.7 Other calf housing – impact

Hutches specifically designed for the paired –rearing of calves were used in this study. As previously mentioned in this thesis (Chapter 1) there are many housing systems that can be used to rear calves. Therefore, how applicable are the results gained from this study to other calf housing systems? This study has shown that calves significantly increase the number of particles in the air as well as the total bacterial count. It would be expected that this finding would also be true in a calf building with calves in individual pens. There would be more particles in the air when there were more calves in the building. The volume of straw used in such a housing system would also be higher than that used in the hutches.

5.5 Conclusion

These results have shown that the presence of calves affects the quality of air in paired-rearing hutches, not only in terms of particulate matter but also in terms of total bacterial count. In the context of the effect of the bedding quality, no conclusive result could be drawn from this study as the straw quality was possibly too similar. Further research would be needed using more extreme and divergent straws in terms of visual quality to allow a more definitive conclusion to be obtained on the effect of bedding quality on air quality. The process of bedding down should be considered as an ‘at risk’ period for air quality in that it introduces more particulate matter and potential airborne microorganisms into the housing environment. It is believed that the findings from this study will be translatable to other calf housing systems.

Chapter 6: General discussion & conclusion

6.1 Introduction

The overall aim of this thesis was to investigate the effect of environmental stressors that are risk factors of BRD (air temperature, wind speed, air quality) on the behavioural reaction and performance of pre-weaned calves. Additionally, the use of a non-invasive technology for the assessment of core body temperature to diagnose signs of disease (an elevation in core body temperature) was assessed.

6.2 Thermal Imaging

The elevation of core body temperature (to 39.5^{o}C and above) is one of the clinical signs of BRD (Knauer et al., 2016; Mahendran et al., 2017a). It is common practice to monitor core body temperature of calves by rectal temperature. However this method is invasive and involves the capture and handling of the calf. In Chapter 2 the concept of using thermal imaging as a technique for assessing core body temperature in calves in on-farm conditions was explored. The idea behind this study was that thermal imaging is a non-invasive method and therefore would eliminate the need for handling the calf to obtain its body temperature. The findings of the study were that there was a low correlation between the maximum temperature obtained from thermal imaging the area in and around the medial canthus of the eye and rectal temperature. The inclusion of air temperature and wind speed along with the volume of milk consumed did not improve the correlation by any great extent. The incorporation of these variables into a predictive equation to predict rectal temperature showed that for rectal temperatures that were associated with pyrexia in calves (≥39.5^{o}C), the equation had a sensitivity of 0% and a specificity of 100%. However, it should be noted that within this limited dataset, there were low number of calves with a rectal temperature ≥39.5^{o}C. Had the use of thermal imaging been proven to provide an accurate diagnosis of the elevation of body temperature when used in on-farm conditions then the technique would have been used for capturing body temperature in subsequent studies. As a result of the study, the technique of thermal imaging was not used in any further studies within this thesis for obtaining body temperature.

Other studies have also found that thermal imaging does not highly relate to rectal temperature. Scoley et al. (2018) explored different anatomical locations on the calf to image. They found a correlation of 0.47 between the maximum temperature from a thermal image of the rectal area and the rectal temperature, which is higher than that found in this study between the maximum temperature from a thermal image of the area around the medial canthus and rectal temperature. However, as Scoley et al. (2018) state obtaining a thermal image of the rectal area involved raising the tail of the calf and handling has been shown to affect the thermal image temperature in cattle (Stewart et al., 2008b). Therefore, if handling the calf anyway, then why not take a rectal temperature? The handling of the calves within Chapter 2 may have influenced the temperature gained via the thermal image.

A similar study to that of Chapter 2 of this thesis has been conducted by Goetz et al. (2020). They also concluded that there was a poor correlation between rectal temperature and thermal image temperature in pre-weaned calves. Therefore, the three studies have all drawn the same conclusion about the relationship between thermal imaging and rectal temperature being poor. The study in Chapter 2 has tried to identify parameters that can assist with the prediction of rectal temperature from thermal imaging temperature. The viability of thermal imaging as a technique to detect an elevation in body temperature is relatively poor and leads us to believe that other methods for picking up signs of disease in a non-invasive manner such as changes in feeding and activity behaviour could be alternatives. Thermal imaging could still prove useful in detecting signs of conditions such as navel ill where the surface temperature of the navel would appear elevated but this would still require a strict protocol for use to obtain an accurate measurement (for example, if the calf was lying down before a thermal image was taken that sufficient time had passed and not in direct sunlight). Thermal imaging has proved to be successful in picking up signs of inflammation such as detecting mastitis through udder skin surface temperature (Colak et al., 2008; Hovinen et al., 2008) and foot lesions that cause lameness (Nikkhah et al., 2005; Alsaaod and Büscher, 2012).

A question that could be raised from Chapter 2 and other studies such as Goetz et al. (2020) and Scoley et al. (2018) is that of what is exactly being identified through the thermal image. Perhaps the thermal image temperatures are identifying a different phenotype such as ill-health rather than an elevation in core body temperature.

The question of automation and the analysis of thermal images should also be explored. If it was possible to implement and install thermal imaging cameras into the milk feed stations of automatic milk feeders, then a much richer overview of the calf could be gained by monitoring how the thermal image temperature changes within and across days as well as the ageing of the calf. Through the implementation of machine learning or artificial intelligence, such techniques may be able to detect changes in the calf better than current analytical techniques.

6.3 Calf housing

It would be fair to say that one of the most commonly asked questions from farmers to calf housing specialists in reference to calf housing is "*what is the best housing for my calves*?" This would imply that somewhere in circulation there is a 'silver-bullet' blueprint for the best design of calf housing that minimises disease and achieves target and above growth rates. The philosophy of some farmers is that if they build a brand new calf building then this will solve all their health and growth issues. However, it is felt that this is the wrong approach to calf housing. The first approach should be based on the basic principles, some of which this study has provided some supporting evidence for. The basic principles of calf housing are to ensure that the housing provides the calf with an environment that is clean, dry and draught-free along with providing adequate fresh air and ventilation to remove particulate matter, moisture and any airborne microorganisms from the air. The practice of good hygiene within the calf housing is also important in the control of disease. The ‘best’ calf house can be badly let down by poor hygiene. Each farm is different in terms of its location, calf management and available resources. Therefore the approach should be “*what is best for my own situation?”*

6.4 Calf responses to housing environment

Chapters 3 and 4 examined the effect of environmental parameters on the response of the calf in terms of behaviour (Chapter 3) and performance (Chapter 4). The results of Chapter 3 showed that calves had an aversion to increasing wind speed and that there was no significant effect of air temperature on the behavioural reactions measured. However, the range of the wind speeds used was quite large (0, 1 and 3.3m/s) and the range of air temperatures was quite narrow (5, 10 and 15^{o}C). It might be that if the range of air temperatures used was wider (e.g. -5^{o}C, +25^{o}C), then the calf may have responded differently. This result provides evidence to support the principle of ensuring that calves are kept in housing that is draught free. The result concerning air temperature highlights the issue raised by Hahn et al. (2013) that the temperature within housing is not important. In turn, this then goes on to support their argument that air temperature alone should not be used as a measure of thermal environment. This argument provides the justification for using effective temperature in Chapter 4 as the measure used to determine whether or not the calves were above or below their LCT at any specified hour of the study. An outcome of Chapter 4 was that the key performance indicator of daily liveweight gain (DLWG) can be influenced by the time the calf has spent below its lower critical temperature (LCT), especially in the first 14 days of life.

If the study in Chapter 3 was to be repeated then it might be beneficial to conduct such a study within a climate chamber, so that there was better control of the air temperature which would allow all calves to be tested under every combination of air temperature and wind speed selected. It would also allow the aspect of relative humidity to be included and controlled for as well. The range of air temperature could be expanded to include lower and higher temperatures than those used; thereby testing calves in so called thermal comfort and under cold and heat stress conditions. As mentioned in the conclusion of Chapter 3, a fascinating concept would be to introduce the technique of operant conditioning, as used by Mejdell et al. (2016), into such a study. In summary, Mejdell et al. (2016) trained horses through the use of symbols to show their preference as to whether or not they required to be blanketed depending on their environmental conditions. Meagher et al. (2015) has shown that such a technique can be successfully used with calves. Therefore, in relation to future work, by using such a technique, the calf could express when climatic conditions were perceived as not being suitable for it and when the use of a calf jacket for protection would be beneficial. The use of calf jackets is a management procedure that is gathering widespread use but the reasoning for their use by some farmers (e.g. improved growth rates in winter months) are not being replicated by the outcomes from the research. Therefore is the marketing of the use of calf jackets slightly misguided? Perhaps their use should be more towards providing short-term protection and additional insulation for young calves against climatic conditions. The calves used in the study for Chapter 4 may have benefitted from having a calf jacket applied during their time in the individual hutch in the early stages of life for this reason. Scoley et al. (2019) examined the behavioural response of calves with and without calf jackets and they showed that there was no difference in lying behaviour between calves that had a calf jacket applied compared to those that did not. The same study also found that when the calf jacket was removed, there was still no difference in lying behaviour between the calves that were reared with jackets applied and those that did not. These findings again highlight what the purpose of calf jackets are. Calves that spent a large proportion of their time in a thermal environment that was below their LCT had a negative DLWG. Therefore these calves had to use what little reserves they had in order to maintain their core body temperature. However, not all these calves lost weight at this stage. It would be of interest to further examine why some calves lost and others gained weight when kept under the same conditions and management. Possibly, the reason could lay with epigenetics such as phenotypic changes caused by the gestation period of the dam (Thompson et al., 2020).

A minor criticism of Chapter 3 by some would be in the selection of wind speeds used to measure the response of the calves, especially 3.3m/s. Some might say that this wind speed was too high and not something that calves would be exposed to. This wind speed is fully justified when the range of wind speeds collected outside the pens in Chapter 4 are noted. The upper wind speed seen in that study, 3.0m/s, shows that this was a realistic selection of wind speeds for the study in Chapter 3.

The longitudinal observational study of chapter 4 concurs with the belief of Hanninen et al. (2003) that studies on the performance of production animals should be conducted in the field as it contains a complex system of thermodynamics. The calves in that study were exposed to naturally occurring air temperatures with varying combinations of relative humidity, solar radiation (although not monitored in the study) and wind speeds. As Hanninen et al. (2003) also states, there are many more factors than air temperature that affect thermal comfort and LCT. This concept is often omitted from literature discussing thermal comfort and LCT and probably goes on to explain why there is such a variation in recommendations for appropriate air temperatures for housing calves as demonstrated in Chapter 1. The review of the literature (Chapter 1) also highlighted the varying interpretation of the terminology.

Other reasons why studies such as that of Chapter 4 should be conducted in the field are that there is a range of housing and management systems which themselves produce variable results not only within system but between systems. The duration of such a study would then pick up any variability experienced across systems due to different seasons of the year and numbers of calves.

6.5 Air quality

The theme of monitoring air quality within calf housing with respect to particulate matter and bacterial count is a relatively novel concept. This could be due to the lack of provision of equipment to monitor such parameters. However, such devices are now portable and usable in commercial farm situations as has been demonstrated by their use in Chapter 5 and by Lago et al (2006) but understandably still with some associated time and cost. However, with respect to the livestock sector, the vast majority of research has been conducted in pig and poultry units and cattle feedlots. This is partially because the outputs of these industries are carefully monitored and margins are tight, and every incremental change which aids health and therefore growth is searched for.

Off the back of the study in Chapter 5 there are many avenues to explore for future work. The initial avenue for further exploration would be to take a further look at the effect of slightly more divergent qualities of straw on the count of particles at the various sizes and also the total bacterial count. It could be argued that the use of more extreme straw as bedding material (e.g. damp, black in colour, full of other vegetation) would not be representative of the type of straw that would be used on commercial farms to bed down calf pens. However, such a study would not necessarily need to be conducted with calves. As a matter of routine, the used bedding material was removed from the hutch to ensure that the samplings for each day were conducted using the same management procedure. The process of removing the bedding on a daily basis is not something that would be done commercially every day. It would be more common for the bedding to be removed once the occupying calf had moved into another pen. The pen would also either be bedded down daily or at regular intervals. The study in Chapter 5 did not top up the bedding and therefore did not have the same build-up of moisture, ammonia or debris that would be experienced in a normal farm situation. Therefore it would be interesting to consider the frequency of bedding and the effect this has on particulate levels and total bacterial counts.

Another interesting approach would be to conduct a study over a longer monitoring period. There are some signs from the results of the study in Chapter 5 that there is a slight increase in particulate levels and total bacterial counts around the time of feeding. The further study would examine these two measurements over the course of a number of days to incorporate feeding into the sample measurements. If such a theory about an increase in the release of particles and microorganisms into the air was proven then this would advocate avoiding any unnecessary visits into the calf housing outwith the period of feeding to avoid this occurring. Another aspect for further investigation would be to take an in-depth look at the species of bacteria in the air and then examine which have entered the respiratory tract of the calf. Even though there may be high counts in some circumstances, it is not known what conditions are favouring such disease causing bacteria.

As a comparison, the bedding material for some calves could be removed daily, whilst the others had bedding topped up at intervals across the study period. The introduction of calves diagnosed with respiratory disease into a study of similar design to that of Chapter 5 would add another aspect to consider in terms of air quality. It would be assumed that a calf diagnosed with respiratory disease would introduce more bacteria into the air, presuming that the cause of the respiratory disease was bacterial.

The study in Chapter 5 used two calves per hutch for those which required the presence of calves. The results involving the presence of calves would start to suggest that the stocking density of pens (number of calves per pen) contributes towards the levels of particulate matter and airborne microorganisms in the air. More calves (for example 4 calves, 8 calves) would be needed per pen to see if this result evolves. Such a study could be carried out using group housing Igloos which could also indicate the maximum number of calves for such a housing system.

6.6 Challenges of monitoring calf housing environment

No matter which sector of the livestock industry it concerns, it has been well highlighted that there is a need to '*measure to manage'*, meaning that unless people measure parameters of interest (for example, liveweight of cattle), the performance of the stock (for example, daily liveweight gain) will not be known. There is a need for this philosophy to be adopted for assessing the housing environment for calves. The continuous recording of air temperature, relative humidity and wind speed on an hourly basis for use within Chapter 4 emphasised the need for this. Although there was a gradual variation between days, it was interesting to see how much within day variation there could be, especially for air temperature. Such variation would not have been observed had the environmental parameters not been continuously measured. The calves involved in this study were being exposed to this variation and had to overcome it by whatever means available. Future work with the data that was generated for Chapter 4 would look at this variation in terms of day and night and apply similar methodology used within the chapter (age related LCT and effective temperature) and look at the effect it has on DLWG. It may also take into consideration the effect of light intensity (lux) as this will impact the environmental temperature, calf behaviour and the potential growth of bacteria.

Within each chapter of the thesis, details about the location and positioning of the environmental monitoring equipment used have been given to show that the recorded environmental parameters were recorded as near as possible to those that the calf was experiencing (calf height) rather than just being somewhere in the building. However, this was not possible with the continuous recording of wind speed within Chapter 4. Had the anemometer that was used in that chapter been placed at calf level then this would have exposed the apparatus to interference by the calves and without video surveillance there would have been no way to accurately identify when this occurred. Without identifying individual scientific reports and papers, there are occasions in the reviewed literature where details about how and where the environmental parameters have been recorded have failed to be reported. Therefore it is unknown whether or not the air temperature and other such measurements relate to those that were experienced by the study calves or by the observer or were just general building conditions. This requirement should be added to guidelines for measuring and reporting calf experimental data suggested by Kertz and Chester-Jones (2004).

This also brings into question as to the appropriate location and height for the measurement of environmental parameters within calf housing. An impromptu attempt at answering this question was carried out as part of a pilot study for Chapter 4 (Appendix B). However, it lacked robustness and repetition (only one pen used) and had multiple failures of recording equipment along with inadequate physical attachment of some of the equipment within the housing. Nevertheless, this exercise did underline some of the challenges and questions surrounding the monitoring of the climatic environment for calves:

- Positioning of recording equipment (see Appendix B) – Sensors to continuously record air temperature and relative humidity were placed in one group housing Igloo pen either inside the Igloo or outside in the straw area at three varying heights (0.3m to represent calf lying height, 0.75m to represent calf standing height and 1.5m to represent an above calf height (referred to as ‘Human’). From the available data, the sensor that was placed at calf lying height and on the left hand side of the pen in the resting/exercise area (outside) had higher relative humidity readings than the other two heights and higher than the right hand side of the pen. This could have been as the result of the close proximity of the sensor to the straw bedding material which had the opportunity to become damp and saturated with calf urine. However, more replication would be needed to see if this was a robust finding. Also, the mean air temperature across all three heights was 1.7^{o}C higher inside the Igloo than outside. Therefore, consideration should be given to recording this parameter in both areas of the pen (inside Igloo and outside) but the attachment of the sensors inside the Igloo proved difficult and would require further investigation into the most reliable method for doing so. The placement of the sensor in relation to the sun should be taken into consideration as well. The sensor should not be placed in direct sunlight as this will not give a true indication of the air temperature.

- Calf experience – A sensor was attached to a collar that was placed around the neck of the calves in the pilot study with the objective of looking at the correlation between sensors placed around the housing pen and that experienced by the calf. This highlighted that the body heat produced by the calf itself and other calves that huddled against the calf with the sensor could influence the recorded measurement. A review of the literature found a study by Borderas et al. (2009) that showed there was a moderate correlation (r=0.49) between the temperature recorded by sensors attached to calves and that from sensors within the building. Results from this pilot exercise were following a similar trend in terms of correlation, but due to the small number of calves used and the interference of other calves we cannot be certain.

As mentioned earlier, the concept of measuring the air quality in calf housing is relatively novel. The ventilation of a building can be monitored through the release of smoke pellets, but this relates to air inlets and outlets not to what is present in the air. As shown by Chapter 5 there are devices available that can measure particulate matter and airborne microorganisms present in the air. However, these are quite specialist pieces of equipment with a considerable financial outlay. Devices such as particle counters may be beneficial to incorporate into the control functions of specialist buildings that are climate controlled. Before this happens, there needs to be industry applied standards as to the level of particles at various sizes that are deemed to be a risk to health and when an intervention is needed.

In conclusion, if academia and industry specialists are advocating the need to '*measure to manage*' the calf housing environment to take informed action, then there is a real need to provide exemplary guidance to the industry evolved through evidence based research.

6.7 Conclusions and recommendations

Overall, it has been shown that calves are affected by their housing climatic environment, especially in the early stages of the rearing phase. The exclusion of draughts allows the calf to remain in conditions it perceives to be comfortable provided that other aspects such as air quality are maintained. Based on the literature, the provision of nutrition will also assist with mitigating the effects of the climatic environment by providing the calf with the opportunity to increase energy intake to enable it to increase heat production.

More emphasis in studies must be placed on the behavioural reaction of the calf towards its perception of the housing environment. The behavioural reaction of the calf will give a strong and sometimes visual indication about how it feels about the environment it is in. Behaviour can also be a useful measure of early signs of disease.

As the realisation of the importance of youngstock management gains momentum amongst academia and industry, a general recommendation would be to create a consortium of stakeholders where the basic principles, such as thermal comfort and LCT, and the associated terminology could be discussed with the aim of having a consensus on the definitions as well as generating material to disseminate to the industry and then everyone is conveying the same information. Also by doing so, there may be a more targeted approach to reducing mortality and incidences of BRD in calves.

Another recommendation would be the sharing of data within academia on similar topics, for example thermal imaging. The combining of such resources could help progress the future development of such a technology through the means of artificial intelligence to develop its predictive capabilities. The aim of research should be to discover and create new possibilities to drive the livestock industry forward into the future and NOT for the personal gain of the academic.

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Appendices

Appendix A

Table A-1 Description of climatic conditions by minutes post straw bedding applied (particle count)

	Minutes post straw bedding application					
	0mins	**40mins**	**80mins**	**330mins**	**370mins**	**410mins**
Wind speed (m/s):						
Mean	0.2	0.3	0.3	0.4	0.5	0.4
Median	0.0	0.0	0.0	0.4	0.4	0.4
Range	0.0, 1.7	0.0, 1.5	0.0, 1.8	0.0, 1.5	0.0, 1.7	0.0, 1.9
Temperature (^{o}C):						
Mean	6.9	7.9	8.7	10.1	9.8	9.2
Median	7.6	8.3	10.0	10.7	10.4	9.5
Range	-4.2, 12.2	-3.6, 13.3	-2.8, 15.6	-1.7, 18.4	-1.4, 19.5	-1.7, 15.8
Relative humidity (%):						
Mean	88.4	84.1	82.2	76.3	77.5	80.3
Median	89.3	83.7	88.3	76.2	77.2	85.4
Range	62.4, 100.0	48.3, 100.0	41.4, 100.0	38.3, 100.0	38.7, 100.0	32.9, 100.0

Table A-2 Description of climatic conditions by minutes post straw bedding applied (total bacterial count)

	Minutes post straw bedding application					
	0mins	**40mins**	**80mins**	**330mins**	**370mins**	**410mins**
Wind speed (m/s):						
Mean	0.2	0.3	0.3	0.2	0.3	0.2
Median	0.0	0.0	0.0.	0.0	0.0	0.0
Range	0.0, 0.8	0.0, 1.5	0.0, 1.0	0.0, 1.1	0.0, 1.5	0.0, 1.3
Temperature (^oC):						
Mean	5.9	6.5	7.3	10.0	9.8	9.2
Median	6.2	7.0	7.5	11.1	11.4	10.8
Range	-1.1, 12.2	-0.8,12.8	-0.5,15.6	2.4, 16.6	1.3, 19.5	-0.6, 15.8
Relative humidity (%):						
Mean	91.0	88.5	86.4	75.3	75.1	77.3
Median	95.8	93.6	93.2	74.3	77.1	79.3
Range	71.2, 100.0	69.8, 100.0	45.4, 100.0	45.0, 100.0	43.8, 100.0	41.2, 100.0

Appendix B

Placement of sensor exercise – Summary

Objective

It is often documented that the UK cattle industry needs to 'measure to manage' with one of the areas often mentioned being the housing environment of calves. However, this is not followed up with guidance on the best practice of how to measure. The objective of this short exercise was to compare the variables of air temperature, relative humidity and water vapour density by place, position and height within the group housing Igloo pen in anticipation of providing some research based evidence for the most suitable locations to measure such parameters.

Materials and methods

The exercise was carried out from 1 March until 7 March 2017 inclusive at SRUC Dairy Research & Innovation Centre, Crichton Royal Farm, Dumfries, Scotland using one group-housing Igloo and pen. The calf housing system comprised of a hand laminated fibre-glass constructed Igloo (3.9m length, 4.4m width, 2.2m height) (*Holm & Laue, Westerrönfeld, Germany*) which provided the calves with shelter. In front of the Igloo there was a resting and exercise pen (5.1m x 5.1m). This exercise and resting area was within a roof structure without exterior walls. The floor of both areas (Igloo and exercise/resting area) was covered in straw, and this was regularly replenished when required.

Sensors and Measurements

Onset HOBO U12-012 Temp/RH data loggers (*Onset Computer Corporation, Bourne, MA, USA*) were used in this study. Each data logger (referred to hereafter as 'sensors') recorded temperature (^{o}C) and relative humidity (%) at 10 minute intervals over the seven days of the study. The data loggers had an accuracy for temperature of ±0.35^{o}C and an accuracy for relative humidity of ± 2.5% between 10 and 90% and ±5% when below 10% and above 90%. They were all uniquely identified so that upon download of the data, their position and height could be retrieved. Before any sensors were mounted anywhere, the sensors were checked for any interference with electrical equipment that already existed within the calf building (e.g. automatic milk feeders). No interference was observed. The sensors in the Igloo and pen but not on the calves were enclosed within a wire mesh structure created to protect the sensors (dimensions of mesh: 1mm wire; 12mm by 12mm squares within) (Figure B-1).

In terms of sensor height and locations, there were five groupings each containing three sensors. Each of the three sensors within a grouping were placed at differing heights: one at 0.3m (representing calf lying height), one at 0.75m which related to calf standing position height, and another at approx. 1.5m which related to the human height relative to the calf (referred to as 'Human'). Examples of sensor placement and heights are illustrated by Figures B-2 and B-3. Three of the three sensor groupings were located within the Igloo, and the other two groupings of three sensors 'outside' in the roofed resting and exercise area. Within the Igloo, one group of three sensors was located to the left of the entrance to the Igloo in an area equidistant between the entrance and the rear of the Igloo and the other adjacent on the opposite side, again equidistant from the entrance and the rear of the Igloo. The third group of three sensors were placed at the rear of the igloo, which was directly behind the entrance of the Igloo. As a result of background unpublished data on calves at SRUC Dairy Research & Innovation Centre, these areas were known to be where the calves frequently used within the Igloo. Data sourced from the sensors that were attached to the areas in and around the group housing Igloo pen are referred to as *SHED* hereafter.

Figure B-1 Example of “sensor” and protective outer casing

Figure B-2 Illustration of sensor placement outside the Igloo (right hand side) and the height of sensor placement in relation to calves ('Human' height sensor missing from illustration)

Figure B-3 Illustration of sensor placement within Igloo (left hand side), showing the differing heights of the sensors

Calves and management

Four pre-weaned purebred Holstein female calves (range: 17 to 23 days of age), within a group of 14 calves of similar age (range of group:7 to 23 days), that were born into dairy herds at SRUC Dairy Research & Innovation Centre, Dumfries, Scotland were used in this exercise as focal animals. For the duration of the exercise they were housed within the same management group in the system described previously. All calves were fed in accordance with current farm practice at SRUC and had continuous access to the automatic milk feeders, fresh water from a water dispenser, fresh straw via racks, and concentrate pellets fed via a trough at the front of the pen. The calves were allowed to access up to 7l per day of reconstituted milk replacer (*Omega Gold*, *ForFarmers, Suffolk, England*; crude protein 23%, crude fat 18%, crude fibre 0.1%, crude ash 8.5%) from the automatic milk feeders and it was fed at a 15% concentration.

Each calf had a HOBO data logger (sensor) attached to the pre-existing collar around their neck that gave the calf access to the automatic milk feeder (*H&L 100, Holm & Laue, Westerrönfeld, Germany*). These data loggers recorded temperature and relative humidity at the same time interval as the other sensors. Data sourced from the sensors that were attached to the calves is referred to as *CALF* hereafter.

Instantaneous scan samples were taken every 10mins for 3 h/d to record the location of each calf within the pen (inside the Igloo, outside the Igloo). For the purpose of locating the calf in relation to the nearest bank of three sensors around the pen, the outside straw exercise/resting area was divided into nine sections and inside the Igloo was divided into three areas. The posture of each calf was also recorded at every scan noting whether the calf was either standing or lying. Over the seven days of the exercise, the three hour/day observation period was carried out at varying times each day e.g. day 1: 1300h to 1600h, day 2: 0400h to 0700h, day 3: 2000h to 2300h in anticipation that observations could be gained under differing ambient conditions.

Data analysis

The temperature and relative humidity data collected from all the sensors (*SHED* and *CALF*) were examined for anomalies. A temperature data point was considered anomalous if there was an increase of 3°C or more from the previous reading. For example, if the temperature reading at 0900h was 7.5°C and the reading at 0900h was 16.0°C, then the reading at 0910h was excluded. The corresponding relative humidity reading at that time point was also excluded.

Water Vapour Density (g/m^3) was calculated retrospectively for each ten minute time-point using the temperature and relative humidity values (Vaisala, 2013). This was done by firstly calculating the Water Vapour Saturation Pressure (P_{ws}):

$$P_{ws} = A \text{ x}10(m \text{ x } T/\ T+T_n)$$

A represented the constant value of 6.116441, m represented the constant value of 7.591386, T_n represented the constant value of 240.7263 and T represented the air temperature (°C). The constant values used were for water and the temperature range of -20 to + 50°C having a maximum error of 0.083%. The values obtained for P_{ws} were subsequently used to calculate Water Vapour Pressure (P_w):

$$P_w \text{ (hPa)} = P_{ws} (T) \text{ x } (RH/100)$$

P_{ws} (T) represented the water vapour saturation pressure at a given temperature (hPa) and RH represented relative humidity (%). The values obtained for P_w were then able to be used for the calculation of Water Vapour Density (WVD):

$$\text{WVD (g/m}^3) = C \text{ x } (P_w / T)$$

C represented a constant of 2.16679 (gK/J) and P_w represented water vapour pressure (Pa).

Descriptive statistics were performed using R software (R Core Team, 2016).

Results

SHED

From a total of 15120 observations for *SHED*, 12913 observations remained following the method for excluding data as previously mentioned. Tables B-1, B-2 and B-3 illustrate the number of observations and descriptive statistics obtained for each data collection point for air temperature, relative humidity and water vapour density respectively. The sensor inside the Igloo on the left hand side at calf lying height failed to record any data. The sensor within the Igloo in the centre at standing height only recorded for one hour.

The air temperature was on average 1.7^{o}C higher inside the Igloo *(In*) compared to outside in the straw area (*Out*) (Table B-4). A similar trend was also found for relative humidity and water vapour density with relative humidity being 6.6% higher *In* compared to *Out* and water vapour density 1.1g/m^3 higher *In* than *Out*.

Table B-1 Descriptive statistics of air temperature (^oC) by placement of sensors

Place	Height	Position	No. obs.	Min	Max	Mean	Median	SD	SE
In	Lying	Left	0	-	-	-	-	-	-
		Centre	980	0.5	17.5	7	6.8	3.14	0.10
		Right	1003	0.8	15.8	7.8	7.9	2.80	0.09
	Standing	Left	1008	1.1	19.3	7.6	7.7	3.17	0.10
		Centre	6	1.4	1.8	1.6	1.6	0.14	0.06
		Right	914	0.8	15.8	7.6	7.8	3.03	0.10
	Human	Left	1008	0.9	20.1	7.5	7.2	3.55	0.11
		Centre	1008	0.2	20.9	7.3	6.8	3.75	0.12
		Right	1008	0.9	16.1	7.3	7.3	2.95	0.09
Out	Lying	Left	1008	0.2	15.1	5.9	5.8	2.73	0.09
		Right	969	0	14.9	5.8	5.7	2.71	0.09
	Standing	Left	1008	-0.2	12.8	5.5	5.5	2.65	0.08
		Right	986	-0.2	14.1	5.7	5.6	2.71	0.09
	Human	Left	1006	-0.1	12.5	5.6	5.6	2.65	0.08
		Right	1001	-0.2	13.7	5.7	5.7	2.68	0.08

Table B-2 Descriptive statistics of relative humidity (%) by placement of sensor

Place	Height	Position	No. obs.	Min	Max	Mean	Median	SD	SE
In	Lying	Left	0	-	-	-	-	-	-
		Centre	980	63.1	93.5	85.3	86.8	6.02	0.19
		Right	1003	47.9	92.7	80.2	82.2	8.62	0.27
	Standing	Left	1008	44.7	93.0	81.7	85.4	9.43	0.30
		Centre	6	84.8	85.7	85.2	85.2	0.26	0.11
		Right	914	47.1	98.6	80.3	83.1	9.50	0.31
	Human	Left	1008	39.8	93.9	82.0	86.7	11.25	0.35
		Centre	1008	42.0	90.1	80.9	85.0	9.48	0.30
		Right	1008	46.3	93.6	82.2	85.4	9.49	0.30
Out	Lying	Left	1008	50.1	90.0	80.9	84.0	7.47	0.24
		Right	969	47.9	81.4	73.5	75.9	5.80	0.19
	Standing	Left	1008	52.3	82.9	75.1	77.5	6.02	0.19
		Right	986	50.9	82.1	74.4	76.8	6.17	0.20
	Human	Left	1006	51.0	81.1	73.7	75.9	6.01	0.19
		Right	1001	49.8	80.1	73.3	75.7	5.78	0.18

Table B-3 Descriptive statistics of water vapour density (g/m^3) by placement of sensor

Place	Height	Position	No. obs.	Min	Max	Mean	Median	SD	SE
In	Lying	Left	0	-	-	-	-	-	-
		Centre	980	4.2	10.4	6.7	6.6	1.20	0.04
		Right	1003	4.7	8.8	6.5	6.6	0.76	0.02
	Standing	Left	1008	4.6	9.7	6.6	6.7	0.90	0.03
		Centre	6	4.5	4.7	4.6	4.6	0.06	0.02
		Right	914	4.6	9.8	6.4	6.5	0.83	0.03
	Human	Left	1008	4.6	10.1	6.5	6.6	0.89	0.03
		Centre	1008	4.2	9.6	6.4	6.4	0.97	0.03
		Right	1008	4.6	8.7	6.5	6.5	0.79	0.02
Out	Lying	Left	1008	4.2	8.1	5.9	5.9	0.74	0.02
		Right	969	3.7	7.3	5.3	5.3	0.69	0.02
	Standing	Left	1008	3.8	7.2	5.3	5.3	0.68	0.02
		Right	986	3.7	7.0	5.3	5.4	0.67	0.02
	Human	Left	1006	3.7	6.8	5.2	5.3	0.65	0.02
		Right	1001	3.6	6.8	5.2	5.3	0.66	0.02

Table B-4 Descriptive statistics for variables by place of sensor (*In, Out*)

Variable	Place	No.obs.	Min	Max	Mean	Median	SD	SE
Temp (^oC)	*In*	6935	0.2	20.9	7.4	7.3	3.23	0.04
	Out	5978	-0.2	15.1	5.7	5.7	2.69	0.03
RH (%)	*In*	6935	39.8	98.6	81.8	85	9.36	0.11
	Out	5978	47.9	90.0	75.2	76.7	6.77	0.09
WVD (g/m^3)	*In*	6935	4.2	10.4	6.5	6.5	0.92	0.01
	Out	5978	3.6	8.1	5.4	5.4	0.72	0.01

Temp – air temperature, RH – relative humidity, WVD – water vapour density

In terms of height, the mean air temperature and water vapour density were consistent across the three heights (Lying, Standing, Human) (Table B-5).

Table B-5 Descriptive statistics for variables by height of sensor

Variable	Height	No. obs.	Min	Max	Mean	Median	SD	SE
Temp (^oC)	*Lying*	3960	0	17.5	6.6	6.5	2.97	0.05
	Standing	3922	-0.2	19.3	6.6	6.5	3.07	0.05
	Human	5031	-0.2	20.9	6.7	6.5	3.26	0.05
RH (%)	*Lying*	3960	47.9	93.5	80	80.7	8.22	0.13
	Standing	3922	44.7	98.6	77.9	78.8	8.54	0.14
	Human	5031	39.8	93.9	78.4	78.6	9.57	0.13
WVD (g/m^3)	*Lying*	3960	3.7	10.4	6.1	6	1.03	0.02
	Standing	3922	3.7	9.8	5.9	5.8	0.99	0.02
	Human	5031	3.6	10.1	6	5.9	1.01	0.01

Temp – air temperature, RH – relative humidity, WVD – water vapour density

Similar to the results from the height of the sensor, mean air temperature and water vapour density were consistent between the left and right hand side (Table B-6). It should be noted that there were fewer readings for Centre as this was only recorded inside the Igloo.

Table B-6 Descriptive statistics for variables by position of sensor

Variable	Position	No. obs.	Min	Max	Mean	Median	SD	SE
Temp (^oC)	Left	5038	-0.2	20.1	6.4	6.3	3.12	0.04
	Centre	1994	0.2	20.9	7.1	6.8	3.47	0.08
	Right	5881	-0.2	16.1	6.6	6.6	2.96	0.04
RH (%)	Left	5038	39.8	93.9	78.7	79.3	9.01	0.13
	Centre	1994	42.0	93.5	83.1	85.9	8.25	0.18
	Right	5881	46.3	98.6	77.3	77.6	8.55	0.11
WVD (g/m^3	Left	5038	3.7	10.1	5.9	5.8	0.97	0.01
	Centre	1994	4.2	10.4	6.5	6.5	1.10	0.02
	Right	5881	3.6	9.8	5.9	5.8	0.96	0.01

Temp – air temperature, RH – relative humidity, WVD – water vapour density

CALF

Only two of the four sensors on calves successfully collected data. In total, 220 observations remained for the analysis of CALF data with associated SHED data. This comprised 65 observations where the calves were inside the Igloo and 155 when the calves were outside in the straw resting/exercise area. Of the 65 observations inside the Igloo, 42 were taken when the calves were lying (Table B-7) and 23 when the calves were seen standing (Table B-8). In terms of the 155 observations outside the Igloo, 83 were taken when the calves were lying (Table B-9) and 72 when they were standing (Table B-10). The mean air temperature and mean water vapour density were higher when recorded from the sensors on calves than from the related sensor in the shed (lying or standing) and the sensor at Human height regardless if inside the Igloo or outside.

Table B-7 Descriptive statistics based on when the calf was observed inside the Igloo and lying down

Location	Variable	No. obs	Min	Max	Mean	Median	SD	SE
Calf (lying)	Temp (^{o}C)	42	13.5	28.3	16.7	16.4	2.35	0.36
	RH (%)	42	40.4	67.8	54.0	53.9	6.16	0.95
	WVD (g/m^{3})	42	5.9	11.9	7.7	7.4	1.29	0.20
Shed (lying)	Temp (^{o}C)	42	5.5	11.1	8.5	9.1	1.43	0.22
	RH (%)	42	70.0	85.7	79.7	80.4	4.04	0.62
	WVD (g/m^{3})	42	5.6	8.5	6.8	6.6	0.74	0.11
Shed (Human)	Temp (^{o}C)	42	5.3	11.9	9.0	9.1	1.80	0.28
	RH (%)	42	61.9	87.5	77.2	79.2	6.70	1.03
	WVD (g/m^{3})	42	5.6	8.2	6.8	6.7	0.63	0.10

Temp – air temperature, RH – relative humidity, WVD – water vapour density

Table B-8 Descriptive statistics based on when the calf was observed inside the Igloo and standing

Location	Variable	No. obs	Min	Max	Mean	Median	SD	SE
Calf (standing)	Temp (^oC)	23	14.1	32.5	17.3	16.0	4.17	0.87
	RH (%)	23	36.4	61.9	51.8	52.8	5.68	1.18
	WVD (g/m^3)	23	6.0	12.6	7.7	7.3	1.52	0.32
Shed (Standing)	Temp (^oC)	23	6.0	11.3	9.1	9.8	1.84	0.38
	RH (%)	23	66.3	80.0	75.8	75.6	3.34	0.70
	WVD (g/m^3)	23	5.7	7.7	6.7	6.8	0.62	0.13
Shed (Human)	Temp (^oC)	23	5.3	11.4	8.9	9.9	2.09	0.44
	RH (%)	23	64.8	82.1	76.2	75.2	4.53	0.95
	WVD (g/m^3)	23	5.6	7.5	6.7	6.8	0.62	0.13

Temp – air temperature, RH – relative humidity, WVD – water vapour density

Table B-9 Descriptive statistics based on when the calf was observed outside the Igloo and lying down

Location	Variable	No. obs	Min	Max	Mean	Median	SD	SE
Calf (lying)	Temp (°C)	83	10.4	30.7	17.1	16.8	4.43	0.49
	RH (%)	83	35.1	72.7	52.3	53.2	7.84	0.86
	WVD (g/m^3)	83	4.8	18.7	7.7	7.4	2.09	0.23
Shed (lying)	Temp (°C)	83	2.5	13.3	7.7	7.9	3.08	0.34
	RH (%)	83	60.9	84.5	74.6	74.1	6.28	0.69
	WVD (g/m^3)	83	4.8	7.3	6.1	6.0	0.83	0.09
Shed (Human)	Temp (°C)	83	2.4	12.4	7.4	7.7	2.93	0.32
	RH (%)	83	58.9	79.6	71.4	70.8	5.62	0.62
	WVD (g/m^3)	83	4.5	6.8	5.7	5.6	0.75	0.08

Temp – air temperature, RH – relative humidity, WVD – water vapour density

Table B-10 Descriptive statistics based on when the calf was observed outside the Igloo and standing

Location	Variable	No. obs	Min	Max	Mean	Median	SD	SE
Calf (standing)	Temp (^oC)	72	9.7	25.0	15.9	15.6	3.42	0.40
	RH (%)	72	26.6	66.7	50.1	51.7	8.06	0.95
	WVD (g/m^3)	72	4.9	8.6	6.7	6.6	0.72	0.08
Shed (Standing)	Temp (^oC)	72	2.5	12.5	7.4	7.4	2.51	0.30
	RH (%)	72	59.8	81.9	71.2	70.7	5.04	0.59
	WVD (g/m^3)	72	4.7	6.9	5.7	5.5	0.62	0.07
Shed (Human)	Temp (^oC)	72	2.6	12.4	7.4	7.5	2.46	0.29
	RH (%)	72	58.9	80.0	70.1	69.6	4.81	0.57
	WVD (g/m^3)	72	4.6	6.7	5.6	5.5	0.58	0.07

Temp – air temperature, RH – relative humidity, WVD – water vapour density

Correlations

In general, there was a better correlation between calf and shed measurements for air temperature than relative humidity and water vapour density apart from when the calf was standing inside the Igloo (Table B-11). Apart from inside the Igloo lying, there was a better correlation between Calf and Shed than there was between Calf and Human for all variables with the exception being WVD outside when the calf was lying down.

Table B-11 Correlations between recording from Calf, Shed and related sensor at human height

Place	Posture	Variable	Calf ~ Shed	Calf ~ Human	Shed ~ Human
In	Lying	Temp (^oC)	0.175	0.286	0.851
		RH (%)	0.037	0.122	0.840
		WVD (g/m^3)	-0.033	0.017	0.892
	Standing	Temp (^oC)	0.169	0.139	0.995
		RH (%)	0.145	0.095	0.964
		WVD (g/m^3)	0.250	0.237	0.984
Out	Lying	Temp (^oC)	0.508	0.506	0.990
		RH (%)	0.448	0.463	0.974
		WVD (g/m^3)	0.371	0.378	0.990
	Standing	Temp (^oC)	0.521	0.508	0.995
		RH (%)	0.371	0.360	0.993
		WVD (g/m^3)	0.533	0.519	0.990

Temp – air temperature, RH – relative humidity, WVD – water vapour density

General comments and recommendations

The objective of this exercise was to investigate a suitable location within a group housing Igloo pen to measure environmental conditions such as air temperature, relative humidity and water vapour density.

The mean difference between the air temperature inside the Igloo and outside the Igloo was 1.7^{o}C. The difference in air temperature between inside the Igloo and outside the Igloo would be considered biologically significant to the young calf. Similar trends were also found for relative humidity and water vapour density in that they were higher inside the Igloo than outside the Igloo. Therefore consideration should be given to recording all these parameters in both areas of the pen. However, the attachment of the sensors inside the Igloo proved difficult and further thought and work into a suitable attachment mechanism would be needed. If unable to make this measurement within the Igloo, the experimenter should be mindful to the fact that the air temperature, relative humidity and water vapour density inside the Igloo will be higher than outside the Igloo. Future work should also consider the number of calves within the housing at the time of recording and examine the effect that this has on these parameters.

To try and ascertain an accurate recording of the environmental conditions that the calf was experiencing, sensors were placed on the existing collar that the calves had around their neck. The placement of sensors on the calves resulted in some interference from the body heat of other calves. Further work would be needed to explore this method for the collection of environmental condition recordings.

An aspect from this short exercise that should be highlighted is the failure of the sensors. The failure of some of the sensors to record was not able to be detected until they were taken down for downloading the expected data. A recommendation as a result of this would be to use a sensor with real-time data flow which would highlight any loss in data signal which could be investigated as soon as possible, minimising data loss.

In terms of the height of the sensor, there was nearly no difference in mean air temperature across the three heights (lying: 6.6^{o}C, standing: 6.6^{o}C, 'Human': 6.7^{o}C). The sensor at calf lying height (lying) resulted in the highest mean relative humidity (80.0%) compared to standing (77.9%) and 'Human' (78.4%). Similar to air temperature, there was very little difference in the mean water vapour density recorded at the three heights (lying: 6.1g/m^3, standing: 5.9g/m^3, 'Human': 6.0g/m^3).

Although from this exercise the correlations were only poor to moderate between the recordings from the sensors on the calves and the relevant sensor within the pen (shed), the correlation was better than that between calf and human height. This would suggest that to obtain data on what the calf is experiencing, then such recordings should be taken at calf height. Taking into consideration that the mean relative humidity was highest when the sensor was placed at calf lying height and that this may have been as a result of moisture from the bedding material, then the sensor should be placed at standing height.

To conclude, from this exercise a recommendation for suitable locations to '*measure to manage*' the environmental conditions within a group housing Igloo pen would be to have sensors placed at approximately calf standing height both inside and outside the Igloo. Another recommendation would be for a further more comprehensive and robust study of similar format to be conducted to further cement these recommendations.

Appendix C

D.J. Bell, A.I. Macrae, M.A. Mitchell, C.S. Mason, A. Jennings, M.J. Haskell (2020) Comparison of thermal imaging and rectal temperature in the diagnosis of pyrexia in pre-weaned calves using on farm conditions. Research in Veterinary Science. Volume 131. Pages 259-265. https://doi.org/10.1016/j.rvsc.2020.05.004

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Comparison of thermal imaging and rectal temperature in the diagnosis of pyrexia in pre-weaned calves using on farm conditions

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ABSTRACT

Measuring core body temperature is used as part of the diagnostic process in assessing the health of animals. Typically in calves, this is carried out using a rectal thermometer which can be time consuming, stressful to the calf and is invasive by nature. A non-invasive technique that is gaining recognition is thermal imaging. This study investigated the use of thermal imaging as a technique to assess core body temperature in pre-weaned artificially reared calves. A total of 125 male and female calves had rectal temperatures measured daily from day 7 until day 40 of life, and at the same time had a thermal image taken of the area around the medial canthus of the eye. A weak correlation ($r = 0.28$) was found between calf rectal temperature and thermal image temperature. A multivariable predictive model for core body temperature increased the correlation ($r = 0.32$) when including the environmental parameters of air temperature ($p < .001$) and wind speed ($p < .001$) as well as reconstituted milk replacer consumption ($p < .01$). The effectiveness of a predictive model including these parameters for the detection of calves with a core body temperature ≥ 39.5 °C was examined and found to have a sensitivity of 0% and a specificity of 100%.

The results of this study demonstrate the need to take thermal environmental parameters into consideration when using thermal imaging to assess body temperature. However, the results suggest that accurate measures of core body temperature using thermal imaging cannot be achieved under commercial farm conditions. Further research is needed to determine what other factors could be measured to increase predictive ability.

1. Introduction

Diarrhoea and respiratory disease are major causes of mortality in artificially reared pre-weaned calves. Early detection of these diseases can result in earlier treatment, thereby reducing their severity for the calf and potentially reducing spread to other members of the group. Body temperature is used as an aid in the diagnosis of ill health in calves (McGuirk and Peek, 2014). Changes in core body temperature can be an early indicator of disease, being one of the first clinical signs to manifest in cases of respiratory disease (McGuirk and Peek, 2014). The most common method for taking body temperature in calves is by inserting a thermometer into the rectum of the animal. This technique requires restraint of the animal, which in itself can act as a stressor. In addition, this method has no agreed technique (Naylor et al., 2012) and is not without errors. One possible source of variation that could cause error is the presence or absence of faeces in the rectum at the time of measurement. As Burfeind et al. (2010) showed, the depth of insertion of the thermometer into the rectum can influence the result gained. Their study found that the temperature obtained at an insertion depth of 11.5 cm was up to 0.4 °C higher compared to temperatures obtained from an insertion depth of 6 cm. The procedure of taking rectal temperature is time-consuming and disruptive to the animal due to its invasive nature, and potentially inaccurate. For these reasons, other techniques to measure core body temperature more easily need to be explored.

A number of novel non-invasive technologies have been developed. Rumen temperature boluses have been developed to record body temperature in cattle (Knauer et al., 2016). However, such a device would still require an invasive procedure (oral administration) to be carried out to enable temperature to be recorded. Also such a device requires a developed rumen and as such would not be of use in new-born calves. FeverTags™ (*FeverTags, 3846 Business Park Drive, Amarillo, Texas 79,110, USA*) have been used to assess body temperature of young and growing classes of bovines in studies by Mahendran et al. (2017) and McCorkell et al. (2014). This technology involves the fixing of the device to the ear and the insertion of a probe into the ear canal to allow

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0034-5288/

for continuous monitoring of body temperature. However, McCorkell et al. (2014) stated that these tags did not always detect sick animals. Suggested reasons for this were the placement of the tag in the ear, as well as the displacement of the ear canal probe. If the tag was placed more laterally in the pinna of the ear, then this reduced the depth of the probe in the ear canal.

A contactless, non-invasive technology that is gaining recognition and momentum in the field is infrared imaging, also referred to as thermal imaging. Thermal imaging devices detect radiated energy from objects, and create electronic images based on how much radiated energy is emitted. The hotter the object is, the more radiated energy is emitted.

This non-invasive technology can be used for assessment of the health and welfare of cattle (Stewart et al., 2005; Theurer et al., 2013). For example, the technique has been used for the detection of lameness (Alsaaod et al., 2014; Alsaaod and Büscher, 2012; Nikkhah et al., 2005) and mastitis in dairy cattle (Berry et al., 2003; Colak et al., 2008; Hovinen et al., 2008) as well as to detect early signs of bovine respiratory disease in growing cattle in feedlots (Schaefer et al., 2012, 2007).

There has been little research carried out investigating the use of thermal imaging in pre-weaned calves. Stewart et al. (2008) used this technique to examine the response of calves to disbudding, and Lowe et al. (2016) have used thermal imaging for the early diagnosis of calf diarrhoea.

The aims of this study were to determine if thermal imaging could be used as an alternative method to assess core body temperature in pre-weaned calves. The correlation between core body temperature (assessed using a rectal thermometer measurement) and the temperature obtained via a thermal image of the medial canthus of the eye was investigated. In addition, a multivariable predictive model was developed that incorporated climatic variables with the aim of improving the ability of the thermal imaging measurements to predict rectal temperature as a proxy for core body temperature.

2. Materials & methods

2.1. Calves – housing and diet

The experiment was conducted at SRUC Dairy Research & Innovation Centre, Crichton Royal Farm, Dumfries between 9 January 2017 and 3 September 2017 (Home Office PPL P204B097E). The study used 100 pure-bred Holstein male and 25 pure-bred Holstein female calves born from the two dairy herds at the Centre.

2.2. Pre-Trial (individual hutch)

All calves were removed from their dam within 24 h of birth, identified by the insertion of eartags, navel dipped with iodine, and fed four litres of thawed high quality colostrum. Once removed from their dam, the calves were transferred to the calf rearing building and placed in an individual calf hutch (*Calf-Tel, Hammel Corporation, Wisconsin, USA*) that was bedded with straw. Female calves were fed in accordance with normal farm practice which was three litres of reconstituted milk replacer (*Omega Gold, ForFarmers, Suffolk, England*; crude protein 23%, crude fat 18%, crude fibre 0.1%, crude ash 8.5%) at a concentration rate of 15% fed twice a day (0730 h and 1600 h) from teat feeder buckets. As part of another research trial, if the male calves still appeared to be hungry (i.e. investigating or licking the teat bucket) after their initial three litres of milk, then they would be offered an additional three litres. This practice was repeated at both feeds during the day. Whilst in the individual hutches, all calves, regardless of sex, had ad-libitum access to fresh water and starter pellets (VitaStart + Deccox, *ForFarmers, Suffolk, England*; crude protein 18%, crude fats & oils 4%, crude fibre 11.5%, crude ash 7%, 3 mm diameter) both of which were fed via two small buckets. All feeding buckets (i.e. water bucket, milk teat feeder bucket, concentrate pellet bucket) remained with each calf for the duration of time that each calf was in the individual hutch. They were then removed and washed before being used for another calf. The individual hutch was also washed between calves and the bedding removed.

2.3. Trial (Group hutch)

Both male and female calves remained in the individual hutches for seven days. They were then eligible to be moved to a group hutch using the Igloo system (*Holm & Laue, Westerfeld, Germany*). The Igloo system consisted a hand laminated fibre-glass plastic constructed dome (igloo) (3.9 m, 4.4 m, 2.2 m) which provided the calves with shelter. In front of the Igloo there was a roofed pen (5.1 m × 5.1 m – length x width). Both areas were covered in straw, and this was replenished on a weekly basis as well as regularly removed (every 2 weeks). Calves were fed milk via an H&L 100 automatic milk feeder (*Holm & Laue, Westerfield, Germany*). Once in the group pen, male calves were allowed to drink up to eight litres of reconstituted milk replacer every 12 h, resulting in a maximum allowance of 16 l per day until the commencement of the weaning process at day 40 of age. There was no restriction on volume per visit to the automatic feeder, thereby allowing the male calves to consume all eight litres in one visit. Female calves had access to 3.6 l every 12 h, resulting in a maximum allowance of 7.2 l per day until day 40 of age, when the weaning process began. However, at every visit to the feeder, female calves were restricted to 1 l per visit. Apart from maximum volume and volume per visit, there were no other differences in the milk feeding process for either sex. All data regarding milk feeding was downloaded from the automatic calf feeder (*H&L Calfguide*). Whilst in the group hutch, calves had ad-libitum access to starter pellets via a trough. This feed was the same as that offered during their time in the individual hutches. Constant access to fresh water was maintained via a drinking bowl. Male calves were housed in a separate group hutch to the female calves but the stocking density of the group pens was kept at 14 per group regardless of the sex of the calf.

2.4. Measurements

Measurements were taken from each calf from the day it entered the group hutch (7.9 days of age ± 0.09) (mean ± se) until day 40 of age when the weaning process commenced. Every day between 0800 and 1000 h, all calves within the group hutch were collected and temporarily penned in the concrete apron at the front of the group hutch (5.1 m by 1.9 m) where they remained until the measurements on all the calves were completed. Each calf was removed from the temporary pen and placed in a modified weigh crate. The modifications to the weigh crate included the removal of the weigh bars and display, and the partial removal of the left panel to allow access to the calf. The modified weigh crate was placed in the alley outside the group hutch. The first procedure carried out on each calf whilst restrained in the modified weigh crate was the assessment of rectal temperature. This measurement was taken using a newly purchased digital thermometer (*Genia Digiflash, St. Hilaire de Chaléons, France*) which was inserted to a depth of 6 cm. The head of the calf was held by an operative to allow a thermal image to be taken by the experimenter. Immediately before the capture of the thermal image, air temperature (°C), wind speed (m/s) and relative humidity (%) were noted from a Kestrel 4500 weather meter (*Nielsen-Kellerman, Birmingham, MI, USA*) with air temperature and relative humidity programmed into the image details. The weather meter was positioned at the level of the calf's head.

2.5. Thermal images

Thermal images were taken using a FLIR SC620 thermal camera (FLIR Comp, Boston, MA, USA) 0.5 m from the subject calf at an angle of between 45° and 90° with an emissivity of 0.98 and a reflective

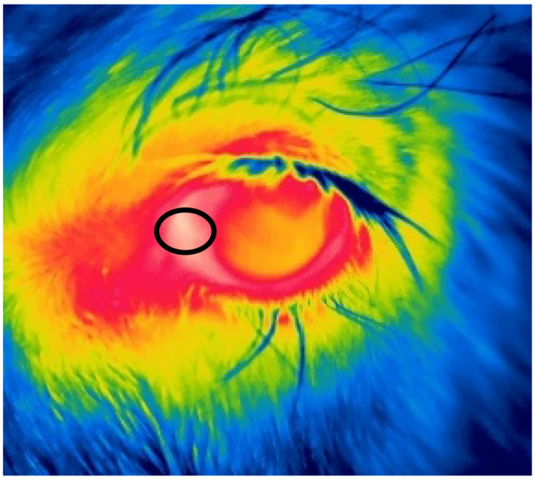

Fig. 1. Thermal image of inner calf eye, black circle indicating area of interest (in and around medial canthus).

temperature of 15 °C. The thermal camera had a pixel resolution of 640 × 480. The thermal image was taken from the area in and around the medial canthus of the left eye of the calf (Fig. 1), as this area of the body has been shown to be a proxy measure for core temperature (Childs et al., 2012). Over the duration of the study, three trained operators acquired the thermal images. The coefficient of variation for the repeatability of thermal imaging and between operator variability was calculated by the following equation: CV(%) = (sd/mean) x 100; and found to be 0.41% and 0.56% respectively. After each imaging session, the thermal images were transferred from the SD memory card of the thermal camera to a computer. Using ThermaCAM™ Researcher Professional 2.10 software (*FLIR Systems, Danderyd, Sweden*), the colour palette was changed from the default Iron to Rainbow900 to allow for clearer observation. An area 2.5 cm in diameter was imposed over the medial canthus area of each image to allow the software to extract the maximum temperature (Byrne et al., 2017) within that area.

2.6. Secondary group examination

During the data analysis, an effect of calf sex on temperature was discovered. However, as female calves were fed on a different regime than male calves, the effect of sex and feeding regime could not be differentiated. Therefore a secondary group of 22 calves were examined further. This non-study group consisted of 10 male and 12 female calves of similar age to those used in this study. Thermal images were taken alongside rectal temperatures using the method explained previously and processed in the same way. The calves examined were all fed on the same milk feeding programme regardless of sex.

2.7. Data analysis

All statistical analysis was conducted using R-software (R Core Team, 2016). On any day, if calves had a missing value for rectal temperature or thermal image temperature, these records were excluded from the dataset for analysis. This resulted in a final dataset of 3737 pairs of rectal and thermal image temperature values from 125 calves. All continuous measures were assessed for normal distribution.

2.8. Water vapour density

Water vapour density (g/m^3) was calculated retrospectively from the recorded air temperature and relative humidity using the following calculations (Vaisala, 2013);

$$A = C\,P_w/T$$

where A represents the absolute humidity (water vapour density) (g/m^3), C represents a constant of 2.16679 (gK/J), P_w represents vapour pressure (Pa), T represents air temperature (K). P_w was derived from the following calculation;

$$P_w = P_{ws}(T) * RH/100$$

where Pw represents vapour pressure (hPa), P_{ws} represents water saturation vapour pressure (hPa), T represents air temperature (°C) and RH represents relative humidity (%). P_{ws} was derived from the following calculation;

$$P_{ws} = A * 10^{(m\,T/T+Tn)}$$

where P_{ws} represents water saturation vapour pressure (hPa), A represents a constant (6.116441), m represents another constant (7.591386), T represents air temperature (°C) and T_n represented the constant 240.7263.

The constant values for A, m and T_n were selected for the temperature range of −20 to +50 °C.

2.9. Statistical analysis

Using the "*lme4*" package (Bates et al., 2015), a multivariable linear mixed effects model to predict core body temperature was constructed by applying the stepwise manual backward elimination method. Due to the repeated measurements of each calf, calf ID was included in the model as a random effect along with thermal image temperature, air temperature, wind speed and reconstituted milk replacer consumption. The maximal model included the variables thermal image temperature, air temperature, relative humidity, water vapour density, wind speed, consumption of reconstituted milk replacer between recording sessions, age of calf and sex of calf. Explanatory variables were then removed in turn, the one with the lowest significance/highest P value first. This was carried out until only variables that were statistically significant *(p value* $<$ *.05*) remained in the model.

2.10. Predictive equation creation

The dataset was randomly divided into two subsets, with half being used to create the model (training dataset, 1859 observations) and the remaining half being used to validate the model created (testing dataset, 1878 observations). The dataset was divided by calf ID (training dataset: 50 male, 12 female; testing dataset: 50 male, 13 female).

The "*lme4*" package (Bates et al., 2015) was used to create the multivariable linear mixed model with the explanatory variables from the previous model refinement. The explanatory variables of air temperature, wind speed and thermal image temperature were centred to allow the model to converge. The training data set was used to generate a predictive model, which was then applied to the testing data set, but excluding the random effects to generate predicted values for rectal temperature. The predicted and actual rectal temperatures were compared.

2.11. Effectiveness of equation

An analysis of specificity and sensitivity was carried out using "*caret*" package to examine how reliable the created equation was at predicting core body temperature over 38.8 °C (median temperature from training dataset) and 39.5 °C that represented pyrexia in calves (Knauer et al., 2016; Mahendran et al., 2017).

3. Results

3.1. Relationship between core body and thermal image temperature

Regardless of the sex of the calf, thermal image temperature was 2.1 °C ± 0.01 (mean ± se) lower than rectal temperature. The mean

Table 1
Description of rectal temperature (˚C) and thermal image temperature (˚C) by sex of calf.

Sex of Calf	Rectal temperature (˚C) (mean ± se)	Thermal image temperature (˚C) (mean ± se)
Male	38.9 ± 0.01	36.7 ± 0.01
Female	38. 7 ± 0.02	36.6 ± 0.02
All	38.8 ± 0.01	36.7 ± 0.01

rectal temperature of the study calves was 38.8 ˚C ± 0.01 (mean ± se) (Table 1). A comparison of the mean rectal temperature by calf sex showed that the rectal temperature of male calves was on average 0.2 ˚C higher than female calves. When plotted, rectal temperature showed a weak positive relationship ($r = 0.28$) with the thermal image temperature (Fig. 2). As the sex of the calf and the feeding regime were confounded in the main study group, the data from the secondary group inspection (where both male and female calves were fed on the same regime) was tested to determine whether a real sex effect existed. Analysis of this data showed that the mean rectal temperature of the calves was 38.7 ˚C ± 0.07 (mean ± se), with the mean rectal temperature for the male calves of 38.8 ˚C ± 0.10 (mean ± se) and the female calves 38.7 ˚C ± 0.09 (mean ± se) ($F (1, 20) = 0.366$, $p = .552$). There was no difference between sexes of calf in terms of thermal image temperature (male calves 37.3 ± 0.12 ˚C; female calves 37.3 ± 0.21 ˚C (mean ± se), $F (1, 20) = 0$, $p = .983$).

3.2. Climatic and diet variables

Air temperatures during data collection ranged from −0.8 ˚C to 22.6 ˚C (10.9 ± 0.007, mean ± se) with wind speed ranging from 0.0 m/s to 2.3 m/s (0.2 ± 0.01, mean ± se). Relative humidity ranged from 49.0% to 100.0% (85.5 ± 0.22, mean ± se) and from the calculation, water vapour density (WVD) ranged from 3.8 g/m^3 to 15.7 g/m^3 (8.8 ± 0.04, mean ± se). Regardless of the sex of the calf, the consumption of reconstituted milk replacer ranged from 0.0 l/day to 15.2 l/day (5.9 l ± 0.04 (mean ± se)). The mean consumption of the reconstituted milk replacer by the male calves was 6.1 ± 0.04 l/day (mean ± se) and 5.1 ± 0.05 l/day (mean ± se) for the female calves.

Table 2
Estimates of effect of variables.

Fixed effect	Estimate	S.E. of estimate	*P* value
Intercept	26.970	0.569	< 0.001
Thermal Image temperature (˚C)	0.336	0.015	< 0.001
Air temperature (˚C)	−0.036	0.011	0.001
Relative humidity (%)	−0.002	0.002	0.486
Water vapour density (g/m^3)	0.026	0.021	0.218
Wind speed (m/s)	0.154	0.022	< 0.001
Sex	−0.177	0.043	< 0.001
Milk diet (litres)	−0.021	0.004	< 0.001
Age (days)	−0.001	0.001	0.713

3.3. Predictive model

The parameters of age, relative humidity and water vapour density were dropped from the maximal multivariable model (*p = .713, p = .486, p = .218* respectively) (Table 2). As a result of this, only the variables of thermal image temperature, air temperature, wind speed, consumption of reconstituted milk replacer (Milk diet) and sex of calf were retained at this stage of the analysis. However, sex of calf was later dropped from the model as a result of the secondary group examination.

3.4. Predictive equation

From the training dataset, the estimates in Table 3 were derived, however these values were based on the centred values for the fixed effects. In the first instance, the values were centred to allow convergence. Centring was carried out by subtracting the mean of the

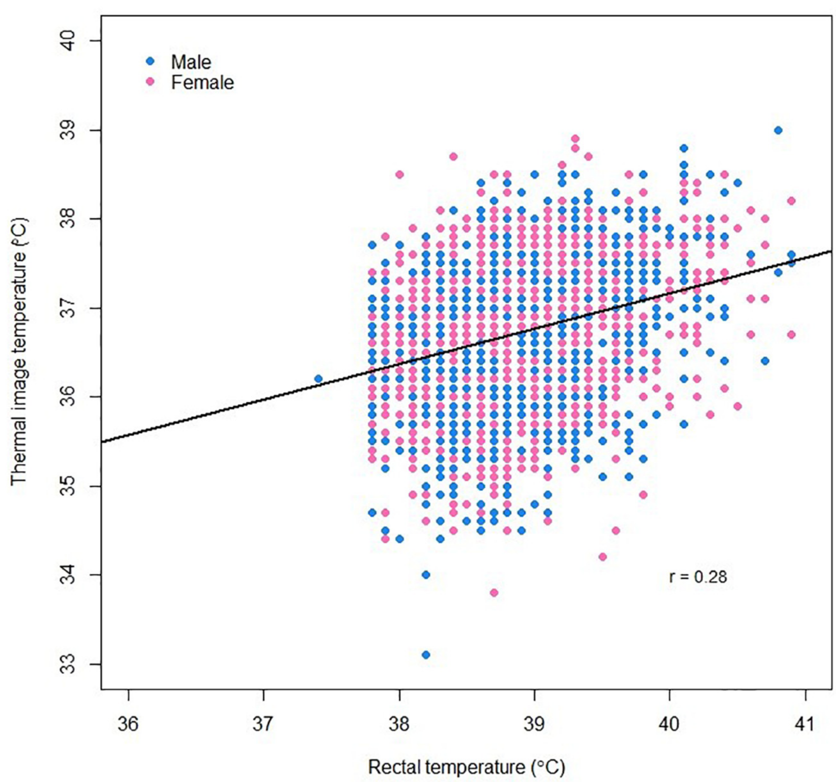

Fig. 2. Correlation of thermal image temperature (˚C) and rectal temperature (˚C) of 100 male and 25 female calves.

Table 3
Estimates of effects of (centred) variables based on training data dataset.

Fixed effects	Estimate	SE of estimate	P value
Intercept	38.823	0.0265	< 0.001
CentImageTemp	0.347	0.034	< 0.001
CentATemp	−0.026	0.006	< 0.001
CentWS	0.122	0.035	< 0.001
CentMilkdiet	−0.020	0.006	0.0002

CentImageTemp – (centred) thermal image temperature, CentATemp – (centred) air temperature, CentWS – (centred) wind speed, CentMilkdiet – (centred) milk diet.

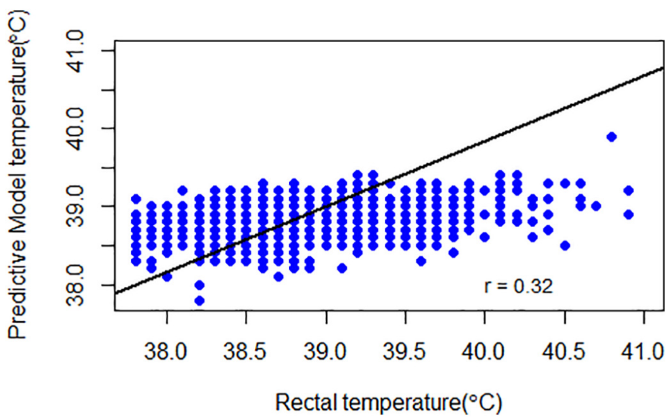

Fig. 3. Correlation of predicted temperature (°C) from created model and rectal temperature (°C) (data from 63 calves).

variable from each of the datapoints of that variable. To obtain the intercept of 26.451 values were un-centred.

The final predictive equation created was:

Rectal temperature = 26.451 + 0.347 *thermal image temperature – 0.026* air temperature + 0.122* wind speed – 0.020*Milk diet.

There was a moderate relationship ($r = 0.32$) between the predicted values generated from the testing dataset based on the model from the training dataset and the actual observed rectal temperatures which is an improvement from the original correlation ($r = 0.28$) (Fig. 3).

3.5. Effectiveness of thermal imaging as a predictor of rectal temperature

Sensitivity (Se) and specificity (Sp) analysis was carried out using the testing dataset to examine how well the model worked at two various temperatures, 38.8 °C (median temperature of the dataset) and 39.5 °C to represent pyrexia (Table 4). The model was less sensitive at predicting rectal temperatures > 39.5 °C than at 38.8 °C (Se = 0.00 (39.5 °C), 0.73 (38.8 °C)). Therefore, it was poor at detecting calves that did in fact have a rectal temperature greater than 39.5 °C (true positive).

Table 4
Sensitivity and specificity analysis of testing dataset at median temperature (38.8 °C) and pyrexia (39.5 °C).

	38.8 °C	39.5 °C
True positive	755	1
False positive	487	0
True negative	350	1664
False negative	286	213
Predictive value positive	0.61	1.00
Predictive value negative	0.55	0.89
Sensitivity	0.73	0.00
Specificity	0.42	1.00

4. Discussion

4.1. Relationship between core body and thermal image temperature

This study aimed to establish if there was a relationship between rectal temperature and the temperature extracted from a thermal image. It was found that there was a weak correlation between rectal temperature and thermal image temperature of 0.28. A similar correlation (0.24) was found by Scoley et al. (2018). George et al. (2014) suggest a much stronger relationship with a correlation of 0.58. A possible reason for the variation in this relationship could be due to the age of animal used in both studies. Calves between 7 and 40 days of age were used in this study, whereas (George et al., 2014) used multiparous cows. Another possible reason for the difference could be due to thermoregulation in the calf. Hill et al. (2016) demonstrated that the body temperature of calves was at its lowest around 0800 h, and at its maximum between 1700 and 2200 h. The study by George et al. (2014) took readings slightly later in the day than this study (0900 to 1200 h, this study 0800 to 1000 h). Also the core body temperature of an adult cow is liable to be more stable than that of a young calf due to the adult cow having a developed rumen which will generate heat.

It has been suggested that rectal temperature is a true measure of core body temperature but it may vary with thermometer placement (Burfeind et al., 2010; Naylor et al., 2012). However, it is the best measure available and is used by veterinary professionals to detect pyrexia. As far as the authors are aware, there is no known agreed procedure for the accurate use of digital thermometers. Likewise it has been shown that eye temperature varies with environmental influences such as wind speed (Church et al., 2014). However, there are no other easily accessible places on the body.

A similar issue is raised when selecting an area of the body to estimate core body temperature with thermal imaging. Teunissen and Daanen (2011) question whether or not the inner canthus of the eye is the most suitable area to take a thermal image of to estimate core body temperature as they found that there was often an inconsistent relationship with the thermal image temperature of this area compared to the temperature of the oesophagus in humans.

4.2. Climatic and diet variables and the predictive model

Both air temperature and wind speed were shown to be of statistical significance ($p < .001$) in the final model to predict rectal temperature using thermal image temperature. These findings corroborate the ideas of Church et al., (2014), who suggested that wind speed affected the thermal image temperature. However, according to Gloster et al. (2011), air temperature had no significant effect on the temperature gained through thermal imaging of the eye ($p > .05$), despite showing a difference between rectal temperature and thermal image temperature of the eye of around 2 °C which is similar to the present study.

From this study, it can be seen that there is a moderate relationship between rectal temperature and predictive rectal temperature using the model created. However, it can also be seen in Fig. 3 that there is large variation surrounding the predicted values that was not captured by the measurements taken in this study. One possible source of variation that was not assessed was the effect of solar radiation on the surface temperature of the eye of the calves. Studies by Paterson et al. (2011) found that levels of solar radiation was statistically significant in their study and included in their model.

Other possible reasons for this variation could be the distance from which the thermal image was taken, the angle at which the thermal image was taken and surrounding environment. Okada et al. (2013) have shown that the distance between the object of interest and the imaging device is of importance to obtain an accurate thermal image temperature. The distance chosen for this study (0.5 m) may possibly not have been adequate. Okada et al. (2013) noted that the greater the distance, the lower the detected temperature by thermal imaging, especially over 2.0 m. This could also account for the lack of agreement in the correlation between thermal image temperature and rectal temperature between this study and George et al. (2014). This study used a distance of 0.5 m whereas George et al. (2014) was slightly closer at 0.3 m. Jiao et al., (2016) have also shown the angle at which the thermal image is taken is also of importance. It was not possible to fully restrain the calf's head during measurements, and therefore the angle at which the thermal image was taken would vary slightly depending on calf movement. This may have resulted in some variability in the reading obtained.

The inclusion of diet in the model would take into consideration that the calf is metabolically immature in terms of temperature regulation. Also environmental and physical stressors can induce metabolic changes in the calf which in turn can affect temperature regulation (Carroll et al., 2012).

4.3. Reliability of model

The sensitivity and specificity analysis suggest that the model could not correctly identifying incidences of pyrexia (i.e. core body temperatures over 39.5 °C; Se =0.00). At this temperature threshold, the model only identified 1 incidence of pyrexia correctly out of the 214. Naturally, the sensitivity of the model improves at a lower temperature threshold (e.g. 38.8 °C) due to more incidences being included in the sample. At the temperature threshold level of 38.8 °C, the model correctly identified 755 incidences above this temperature out of 1041. However, at the 39.5 °C threshold, the predictive equation could correctly exclude the condition (i.e. identify a temperature below 39.5 °C) when it was not present (Sp = 1.00). This can also been seen by the negative predictive value (NPV) (0.89) meaning that 89% of cases truly had a core body temperature below 39.5 °C. The NPV will increase and the PPV will decrease as the prevalence of the condition decreases. Using the 39.5 °C threshold, the mean daily prevalence of pyrexia in the calves used in this study was 10.2% ± 0.56 (mean ± se) and whilst there are no UK figures available on the prevalence of pyrexia in calves, the authors' consider that the sample population was typical of UK dairy calf populations. Therefore, this would suggest that with the incorporation of the identified parameters, thermal imaging could potentially be used to identify a calf with a core body temperature that is not deemed pyrexic.

Despite the apparent positive aspects of the use of thermal imaging such as the non-invasive nature of the technique, it does not appear to be at a stage of functionality where it can be used to detect pyrexic calves reliably. Further work should investigate other potential sources of variation that could help improve the correlation between core body temperature via rectal temperature and thermal image temperature.

5. Conclusion

Based on the results from this study, using a model that includes thermal image temperature and corrected for the environmental parameters of air temperature and wind speed as well as diet, there was a slight improvement in its correlation with rectal temperature from the original correlation of thermal image temperature and rectal temperature. Further investigation under farm conditions would be needed to explore the possibility of improving the relationship. However, from the sensitivity and specificity analysis, there is the possibility that thermal imaging could be used as a “proxy measure” of body temperature to identify non-pyrexic animals.

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