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Studentship Project: Final Report

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Project Title:	**Preventing respiratory disease in calves: ventilation, thermal comfort and disease transmission**		
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CONTENTS

1 Abstract

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. However, there is increasing evidence that there is a conflict between providing adequate air flow for good respiratory health and the prevention of cold stress in calves and/or wet conditions.

The research questions addressed by this research are:

1. Is thermal imaging a reliable way to measure core body temperature in calves?
2. Do calves respond differently to varying air temperature and wind speeds?
3. How long do calves need to be exposed to low temperatures before it negatively impacts on their daily liveweight gain?
4. Does bedding material of varying quality directly impact on the air quality in calf housing?

Key findings:

1. A weak association between rectal temperature and the temperature obtained from thermal imaging the area surrounding the inner eye was found. 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.
2. 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 assessed. Calves showed an aversion to increasing wind speed and that there was no significant effect of air temperature on any of the behavioural measures.
3. 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.

4. 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 consists, amongst other things, of 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.

In conclusion, these studies provide additional 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.

2 Introduction

The female calves born today are the milking and suckler herd of tomorrow and along with the male calves, the future beef production. 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 prospective. 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) stated that from their survey on Norwegian dairy herds, calf mortality rates were 4.6% amongst live-born calves. Overall, it can be seen that the UK has room for improvement in terms of calf mortality.

Enteric (e.g. diarrhoea) 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 report will be based around bovine respiratory disease (BRD), with the main focus surrounding the calf housing environment.

2.1 Bovine respiratory disease (BRD)

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. 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 in the lungs but the classical clinical signs (described later on) are present. BRD is known as a multifactorial disease. This is because numerous environmental and management risk factors (stressors), along with infectious agents (e.g. *Mannheimia (Pasteurella) haemolytica, Mycoplasma bovis, Respiratory syncytial virus (BRSV), Parainfluenza virus III (PI3),*

Bovine viral diarrhoea (BVD)) contribute to the development of disease. The main environmental risk factor relevant to respiratory disease is related to housing.

2.2 Calf housing

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 UK, dairy bred 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). These systems can sometimes be as part of a purpose built facility (i.e. sole purpose of calf rearing) or within an existing building used previously for other purposes (e.g. milking shed (ship-in/byre)). Despite all the various systems to house calves, 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.

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. An example of this statement would be if a farmer/calf-rearer closed the door of the calf house as they perceived it to be cold and the doorway was the only source of fresh air into the calf house, thus creating a build-up of stale air. 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.

2.3 Thermo-neutrality and thermal comfort

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 stressful 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 (Carstens, 1994; Rowan, 1992; Vermorel et al., 1983). 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. The TNZ can be thought of as having three zones within it: cool, thermal comfort and warm (Figure 1).

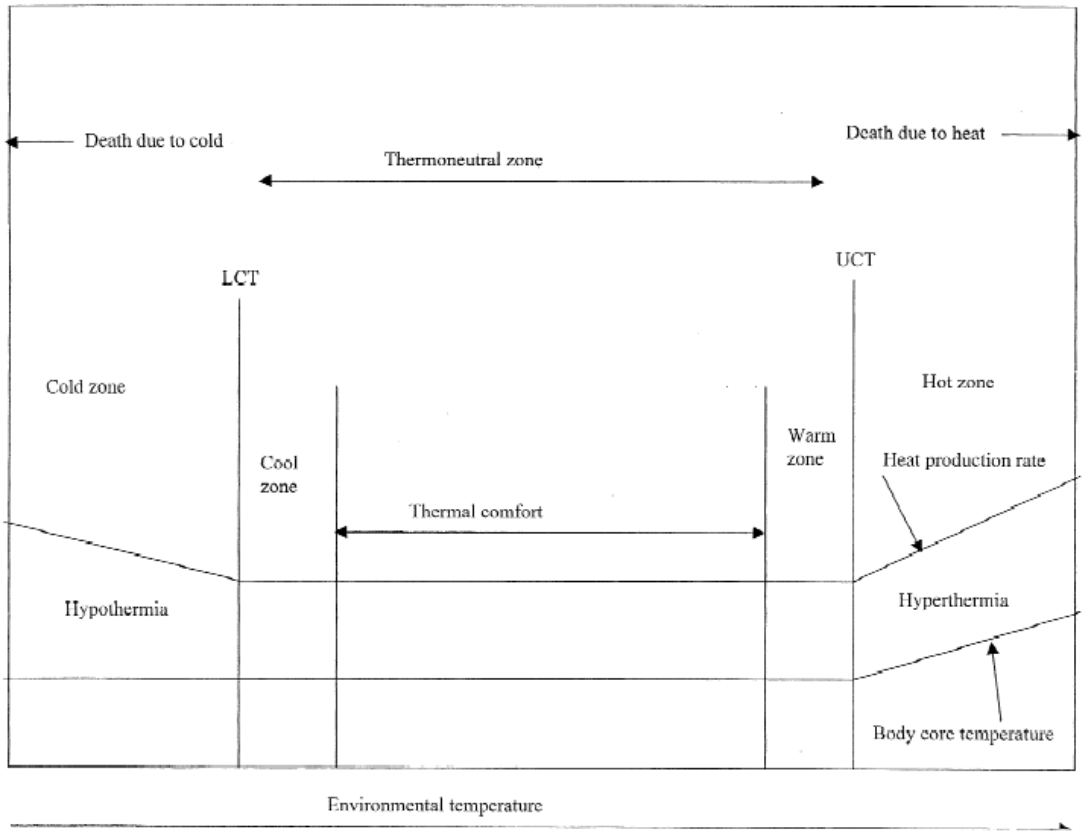

Figure 1 Illustration of thermal zones (source: Kadzere et al., 2002)

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).

2.4 Lower critical temperature (LCT)

Environmental temperatures in the UK will not always be ideal for calves. At present, it is more common for air temperatures to be below that of the temperature range of thermal comfort mentioned by (Stull & 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 & 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 12.8ºC at day 3 of life to 8.2ºC 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 13ºC. Work by (Holmes & 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 shows, as air speed increases, the LCT of the calf, regardless whether it is a new-born or older calf, increases too.

Table 1 Effect of air speed on lower critical temperature (LCT) (source: Webster, 1981)

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

This is as a result of an increasing air movement dislodging the external insulation that the hair coat provides the calf (A. J. F. Webster, 1974). Moisture also has the same effect on calves. Table 1 and the work by (Gonzalez-Jimenez & Blaxter, 1962) also show that the age of the calf can affect the LCT. As the work by Gonzalez-Jimenez & 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.

It should be highlighted that there is some variation in the scientific literature and guidance on calf housing that is freely available to farmers as to the range of environmental temperatures that are considered the TNZ, TCZ and LCT. Much of this information omits the effects of age and nutrition as well. However the effect of age and nutrition is possibly where the variation is resulting from but not emphasised. It is felt that this is an area of slight concern and where industry agreement is needed. Also there is some thought on the importance of air temperature, with Roe (1982)stating that it is relatively unimportant. Hahn et al. (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 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.

2.5 Air quality

As previously mentioned in section 2.2, 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. 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 (Roe, 1982; Turnbull, 1980). 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).

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; Davison et al., 2019; Kaasik & Maasikmets, 2013; Papanastasiou et al., 2011). There is no known published scientific literature with regards to particulate matter and calf housing.

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.

2.6 Objectives & hypotheses

Objective 1: To assess the use of thermal imaging as a way to measure core body temperature in calves (*thermal imaging*).
Hypothesis - Thermal imaging can be used as a proxy for monitoring calf body temperature, and therefore be used as a reliable method to detect BRD.

Objective 2: To assess the effects of climatic conditions on the behavioural response of the pre-weaned calf (*Behavioural response to air temperature and wind speed*) as well as the performance of the calf in terms of daily liveweight gain as a measure of growth (*Calf performance & environment*).
Hypothesis - Calves exposed to adverse environmental conditions (low temperature and high wind speed) would seek to remove themself to a better environment and that exposure to such adverse environmental conditions would result in lower daily liveweight gain.

Objective 3: To examine the effect of a specific management decision, such a provision of bedding material of varying quality, on the quality of the air in calf housing (*Air quality*).
Hypothesis - Poorer quality bedding material would release more particles into the air increasing the potential risk of BRD.

3 Materials and methods

3.1 Calves & management

All the studies presented in this report were conducted at SRUC Dairy Research & Innovation Centre, Crichton Royal Farm, Dumfries, Scotland and used calves sourced from the on-site dairy herd. Unless stated, all calves were managed in accordance to normal SRUC farm management. In summary, once born, calves received four litres of thawed pasteurised, quality tested colostrum via an oesophageal tube and were removed from their dam as soon as possible and within 24 hours and was placed in a straw bedded individual hutch within the main calf rearing building at Crichton Royal Farm. All calves were weighed upon entering the individual hutch ('birthweight'). When housed in the individual hutches, all calves received three litres of reconstituted milk replacer (23% crude protein, 18% crude fat, fed at 15% concentration) twice daily fed via a teat bucket and had ad-lib access to water and starter pellets (18% crude protein, 3mm diameter) from Day 1 of life. Once deemed strong and healthy enough, calves were then transferred into a group housing Igloo pen, normally between 6 and 14 days of life. Each group housing Igloo pen consisted of 14 calves (a mixture of dairy and dairy-beef crosses and male and female calves). Calves were fed via an automatic milk feeder whilst in the group housing Igloo pens and had 7

litres available daily (23% crude protein, 18% crude fat, fed at 15% concentration) until Day 40 of life when the weaning process began. Calves had ad-lib access to starter pellets (18% crude protein, 3mm diameter) (fed via a trough) and fresh water (from a water dispenser) along with straw which was present in racks within each pen.

3.2 Thermal Imaging

This study involved the daily monitoring of 100 dairy (Holstein) male calves and 25 dairy (Holstein) female calves from entering the group housing Igloo pen until the start of the weaning process. The female calves followed normal SRUC farm management (as mentioned above), whereas the male calves were managed in accordance with another AHDB funded study (Enhanced monitoring systems for improved health management of dairy-bred beef youngstock (61100015)). All calves on study had a rectal temperature taken daily using a digital thermometer (*Genia Digiflash, St. Hilaire de Chaléons, France*) which was inserted to a depth of 6cm into the rectum.

Thermal images were taken immediately post rectal temperature 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), as this area of the body has been shown to be a proxy measure for core temperature (Childs et al., 2012). Full description of the materials and methods for this study are described by Bell et al. (2020).

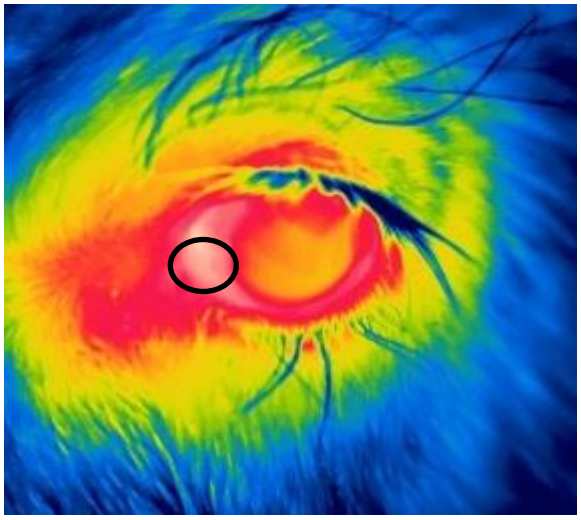

Figure 2 Thermal image of inner calf eye, black circle indicating area of interest (in and around medial canthus)

3.3 Behavioural response to air temperature & wind speed

This study was conducted between 3 April to 18 May 2017, 8 October to 22 November 2017 and 3 April to 26 May 2018 and utilised eighteen pre-weaned Holstein female calves. All calves were tested in the age range of 7-25 days of life, 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.

3.3.1 Test conditions

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 ± 2.0^{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).

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 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.

3.3.2 Test arena

The test arena (Figure 3 & Figure 4) was created in part of the existing calf rearing shed, 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 (1.25m x 2.5m) which were adjacent to each other and could be accessed from the other.

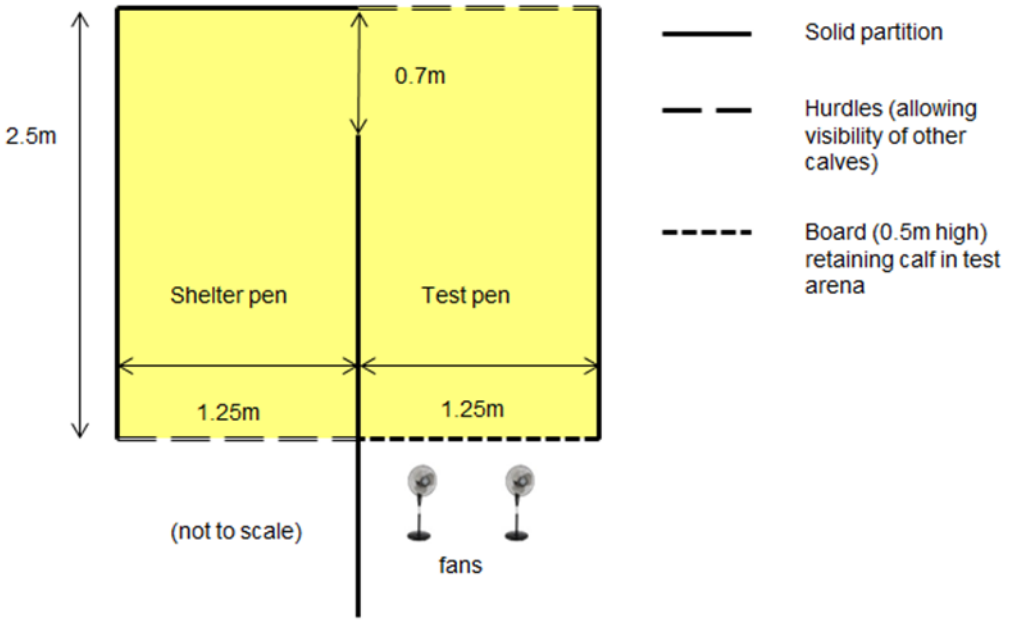

Figure 3 Layout of test arena

Figure 4 Illustration of test arena with calf in test pen and shelter pen adjacent

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. 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. Both parts of the test arena

were bedded with approximately the same amount of straw at each test session. The calf had sight of other calves in the shed at all times during each test session.

3.3.3 Testing process

Before any of the calves were tested, they each received 2 training sessions before the 1st test session so that the calves became familiar with all parts of the test arena.

Prior to each test session commencing the calf was weighed which was used to calculate body surface area. The calf was also visually health assessed using the Wisconsin Calf Health Scoring System (McGuirk, 2008) which included obtaining the rectal temperature of the calf. Any calf with a rectal temperature of 39.5°C and above was not tested as were calves with an overall Wisconsin health score of 5 and above where the rectal temperature was less than 39.5°C. The mean Wisconsin health score for the study was 1.7 ± 0.09 (mean ± se).

The test subject calf 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 and after 20mins had elapsed, recording of the test session was stopped. 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.

3.3.4 Measurements

As well as location (test pen or shelter pen), the following reactive behaviours were recorded: ear flicking, tail flicking, head shakes, whole body shakes, head/ear rubbing and self-grooming. 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 & 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 any 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.

3.4 Calf performance & environment

This longitudinal, observation study was conducted between July 2018 and July 2019 and followed 299 eligible calves from birth (Day 0) until approximately Day 28 of life. Both male and female dairy and dairy-beef cross calves were used in this study.

3.4.1 Climate data

Air temperature (°C), 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.

3.4.2 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 (°C), 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.

Calves were weighed when they were removed from their dam using a manually operated calibrated weigh crate. This weight was referenced as 'birth weight'. 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).

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

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 (Sherwin et al., 2016; Tolley et al., 1988).

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 & 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 12. 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 2. 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).

Farm-staff 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).

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. 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.

Table 2 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

3.5 Air quality

This study was conducted between 14 January 2019 and 16 April 2019, and 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).

(i) (ii)

Figure 5 Layout of study hutches (front (i) and rear (ii) view)

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 that acted as security fencing. 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).

3.5.1 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.

3.5.2 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) due to its 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 3). 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. 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.

A representative sample of each bale was taken and 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 (straw ME) of each straw as 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 4 displays the visual and analytical variation on the two straw beddings chosen and used in this study and Figure 6 illustrates the two straw beddings. Straw 2 was considered to be more lignified than Straw 1 due to the lower ME content. From the assessment and analysis, Straw 2 was deemed of poorer quality than Straw 1.

Table 3 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

Table 4 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 6 Illustration of the two straws used as bedding material in the study

Sampling

3.5.2.1 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 8). 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 7). A sampling rate of 2.83l of air/60s was used in this study.

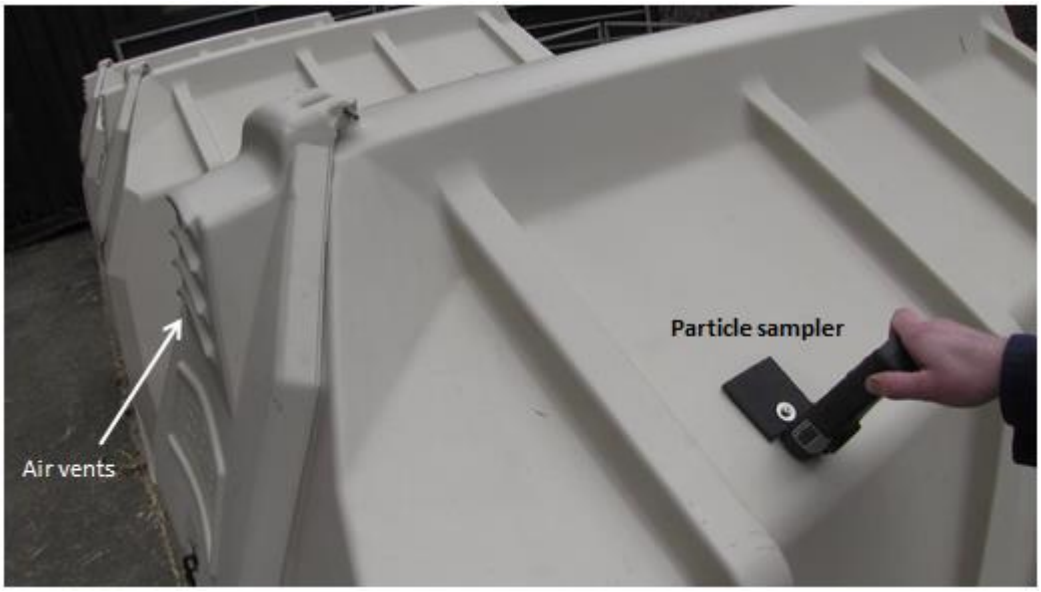

Figure 7 Placement of particle sampler in relation to rear air vents of hutch

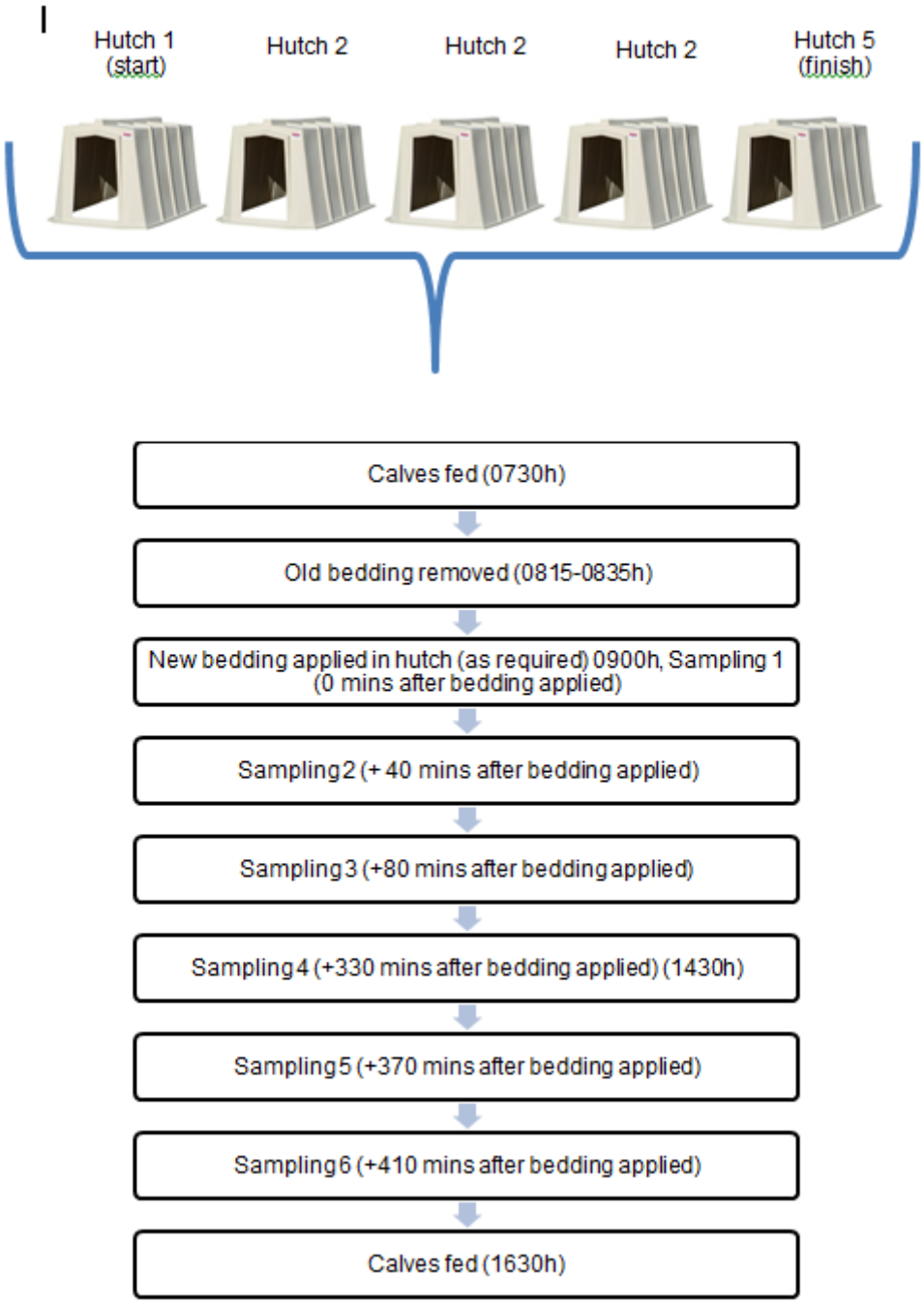

Figure 8 Sampling timeline

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.

3.5.2.2 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>*

4 Results

4.1 Thermal imaging

4.1.1 Relationship between thermal image temperature and rectal 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 rectal temperature of the study calves was 38.8°C ± 0.01 (mean ±se). 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 (Figure 9).

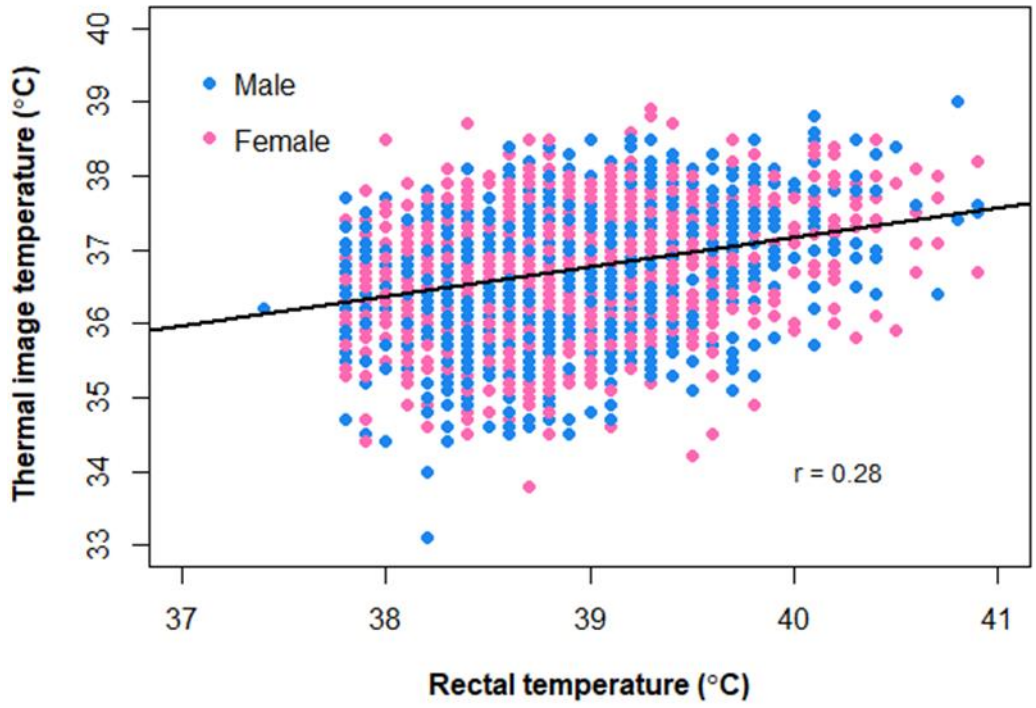

Figure 9 Relationship between thermal image temperature and rectal temperature for 100 male and 25 female calves

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 effect existed. Analysis of this data showed

that the mean rectal temperature of the calves was 38.7ºC ±0.07 (mean ±se), with the mean rectal temperature for the male calves of 38.8ºC ±0.10 (mean ±se) and the female calves 38.7ºC ±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 ±0.12ºC; female calves 37.3 ±0.21ºC (mean ±se), F (1, 20) = 0, p=0.983).

4.1.2 Climate and diet variables

Air temperatures during data collection ranged from -0.8 ºC to 22.6 ºC (10.9ºC ±0.007, 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 consumption of reconstituted milk replacer ranged from 0.0l/d to 15.2l/d (5.9l ±0.04 (mean ±se)). The mean consumption of the reconstituted milk replacer by the male calves was 6.1l/d ±0.04 (mean ±se) and 5.1l/d ±0.05 (mean ±se) for the female calves.

4.1.3 Predictive model & equation

The parameters of age, relative humidity and water vapour density were dropped from the maximal multivariable model (p=0.713, p=0.486, p=0.218 respectively). 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. 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) (Figure 10).

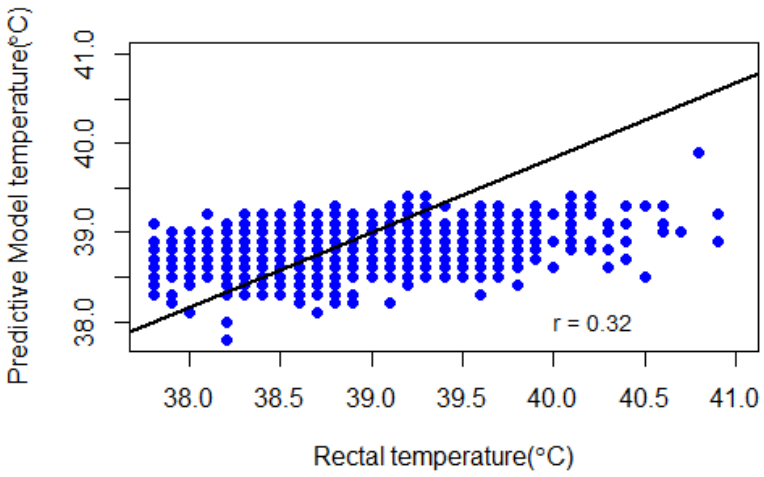

Figure 10 Relationship of predicted temperature (ºC) from created model and rectal temperature (ºC) (data from 63 calves)

4.2 Behavioural response to air temperature & wind speed

4.2.1 Effect of wind speed

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)). There was no significant effect of 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)). 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).

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)). 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*). 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)). There was a significant effect of 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).

4.2.2 Effect of air temperature

There was no significant effect (p>0.05) of air temperature on any of the dependent variables when wind speed was included in the model. In the absence of wind (i.e. data collected at 0.0 m/s, 'still'

conditions), there was also no significant effect (p>0.05) of air temperatures on PropTP, Lat1Place, TbehTP or Lat1React. The results show that there is little evidence to suggest that temperature alone was affecting the behaviour of the calf.

4.2.3 Other variables

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 or age of the calf on PropTP, Lat1Place, TbehTP or Lat1React when wind speed and temperature were included in the model or in the absence of wind (p>0.05).

4.2.4 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, 066 (66%) ± 0.034 for >1.15m^2 (mean ± se). 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). 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)). 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 or Lat1React (p>0.05 respectively). There was also no significant effect of body surface area on the four dependent variables in the absence of wind (p>0.05).

4.3 Calf performance & environment

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, 85 in spring, 54 in summer and 80 in autumn.

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.

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 euthanised 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 were unable to be treated) 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 5 Description of calf related parameters 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).

Table 5 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, CMR – calf milk replacer

4.3.2 Climate – descriptive statistics

Over the course of the study, there was a range of climatic conditions experienced (Table 6). 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.

Table 6 Description of climate parameters

Parameter	Mean	SD	Median	Minimum	Maximum
[1] Air temperature (°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 (°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*

4.3.3 Daily liveweight gain – Birth to group pen *(B2G)*

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 11). 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.

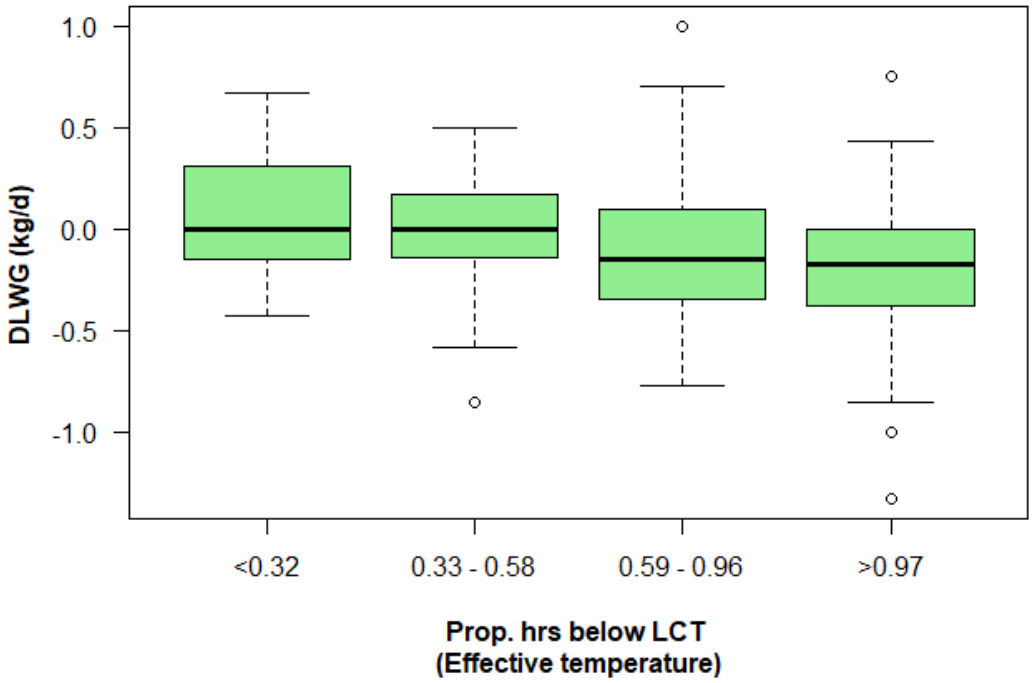

Figure 11 Effect of proportion of hours below LCT on daily liveweight gain (DLWG, Kg/d) for the period between birth and leaving the individual hutch (B2G)

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)

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 12). The more CMR consumed by the calf, the higher the growth rate achieved.

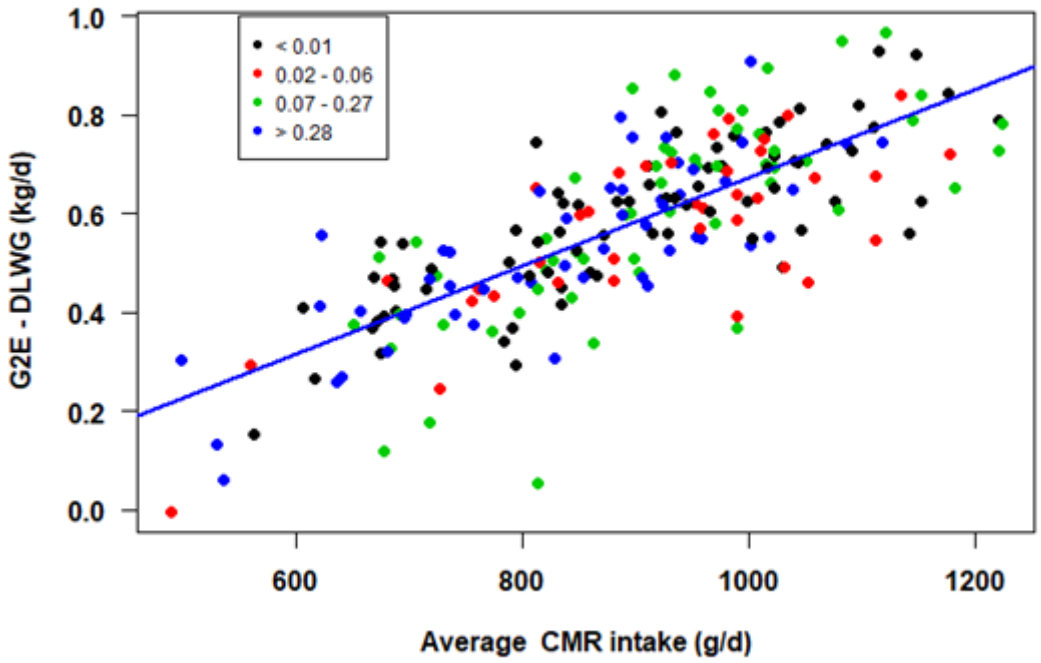

Figure 12 Association of average CMR intake (g/d) on daily liveweight gain (DLWG, Kg/d) between entering the group housing Igloo pen and end of study period (G2E) by proportion of hours below LCT

4.4 Air quality

4.4.1 Effect of Straw Bedding

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 13).

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).

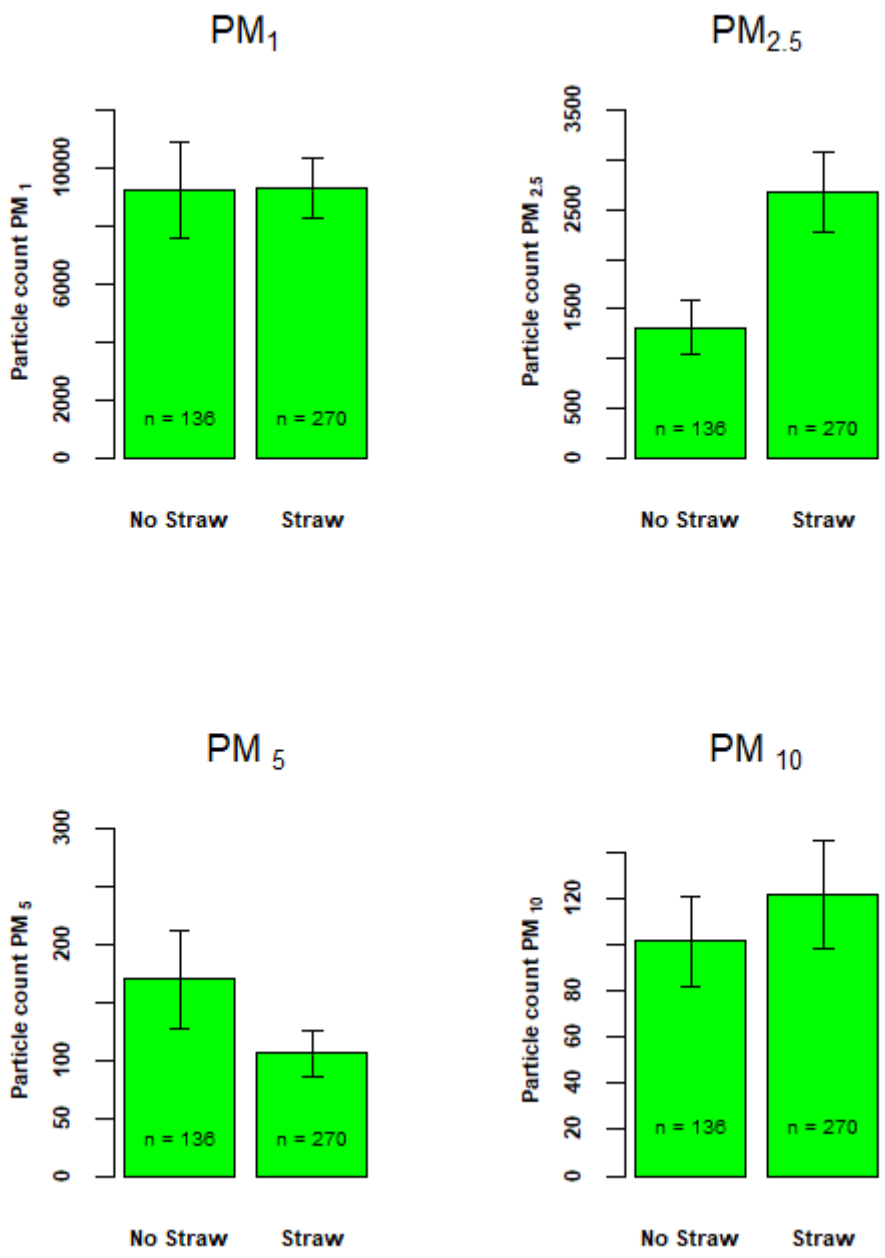

Figure 13 Effect of straw bedding (no straw, straw) on count of particles per particle size (n= number of observations)

In terms of total bacterial count, there were more colonies grown when there was straw bedding in the hutch (Figure 14). There was a significant effect of the presence of straw bedding on the total bacterial count based on the model used (p=0.018).

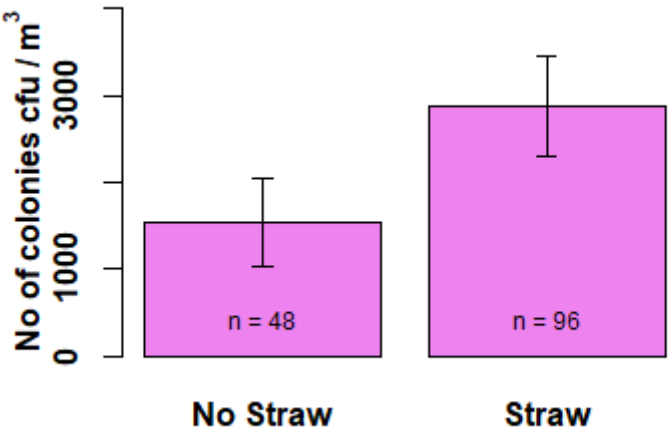

Figure 14 Effect of straw bedding (no straw, straw) on total bacterial count (cfu/m^3) (n= number of observations)

4.4.2 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.

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).

There were slightly more colonies grown from the air samples taken from the hutches containing Straw 2 compared to those air samples taken from hutches containing Straw 1 (Straw 1: 10910.3 cfu/m^3 ± 3580.49; Straw 2: 11521.8 cfu/m^3 ± 1957.43 (mean ±se). However, there was no significant effect of straw quality (Straw 1, Straw 2) on the total bacterial count (cfu/m^3) (p=0.360).

4.4.3 Effect of Calves

The presence of calves increased the count of particles in the air which was consistent across the particle sizes (Figure 15).

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).

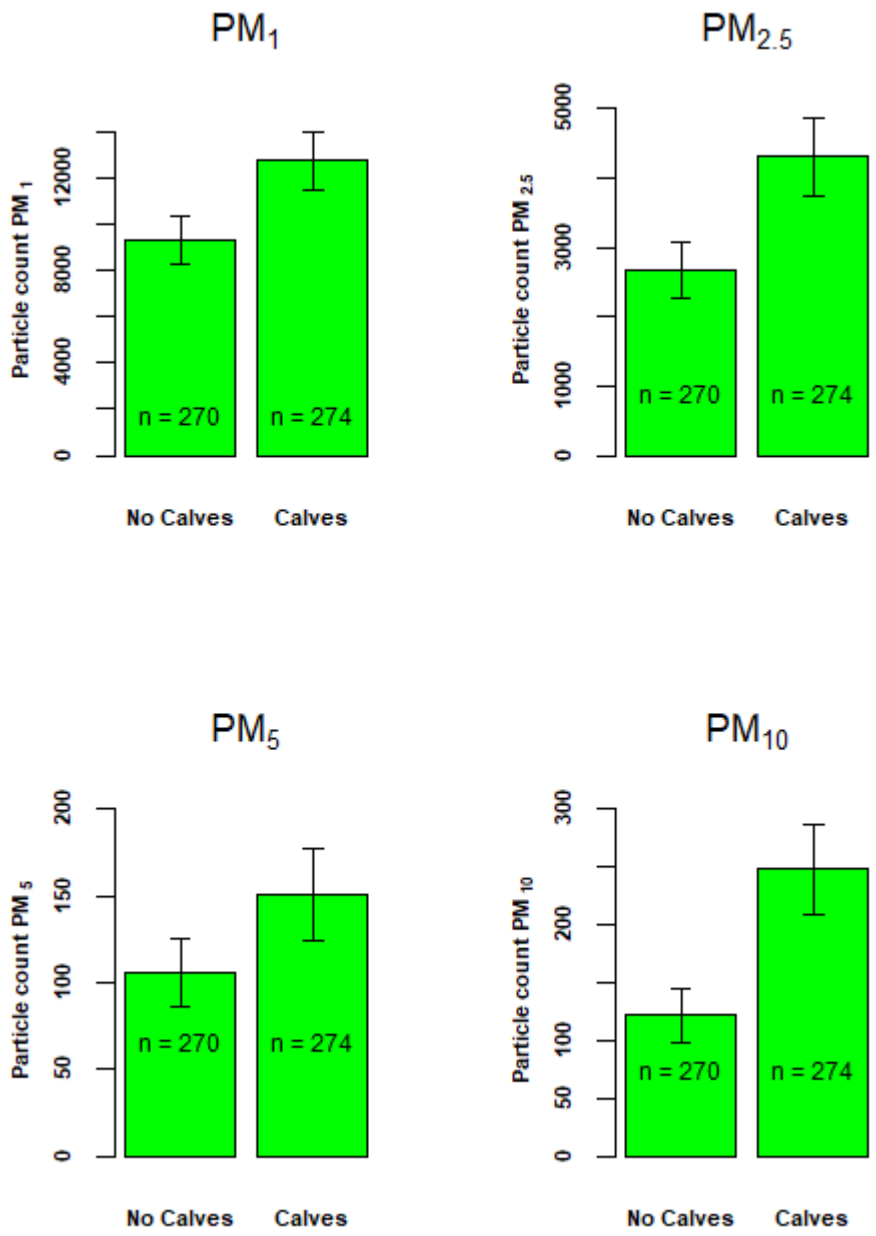

Figure 15 Effect of calves (no calves, calves) on count of particles by particle size (n= number of observations)

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 16). There was also a significant effect of the presence of calves on the total bacterial count (cfu/m^3) ($p<0.001$).

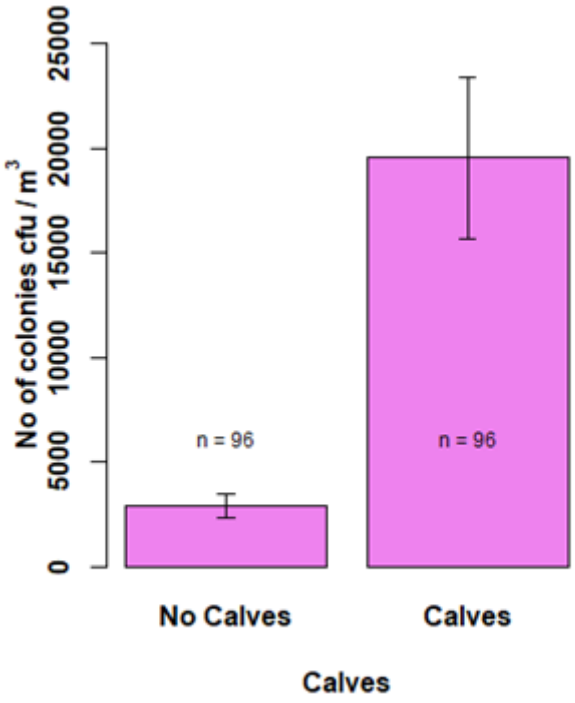

Figure 16 Effect of calves (no calves, calves) on total bacterial count (cfu/m3) (n= number of observations)

4.4.4 Time after straw bedding applied

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 compared to 0mins when examining both the presence of straw (No Straw v Straw) and calves (No Calves v Calves, Figure 17). 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 ($p>0.1$).

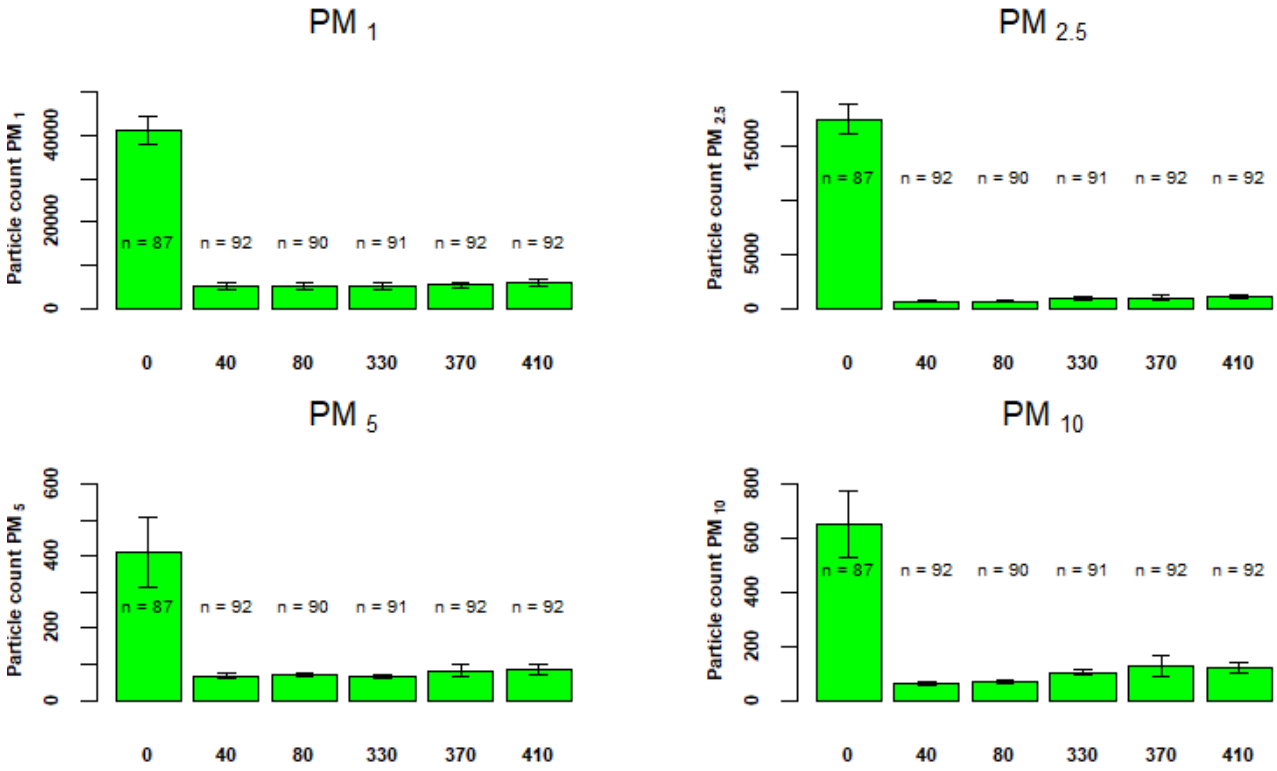

Figure 17 Effect of time after bedding applied (0, 40, 80, 330, 370, 410 mins) on particle count by particle size when examining the presence of calves (n= number of observations)

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. There was no significant effect of the time after the bedding was applied on MPNC (p=0.069).

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 18). 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).

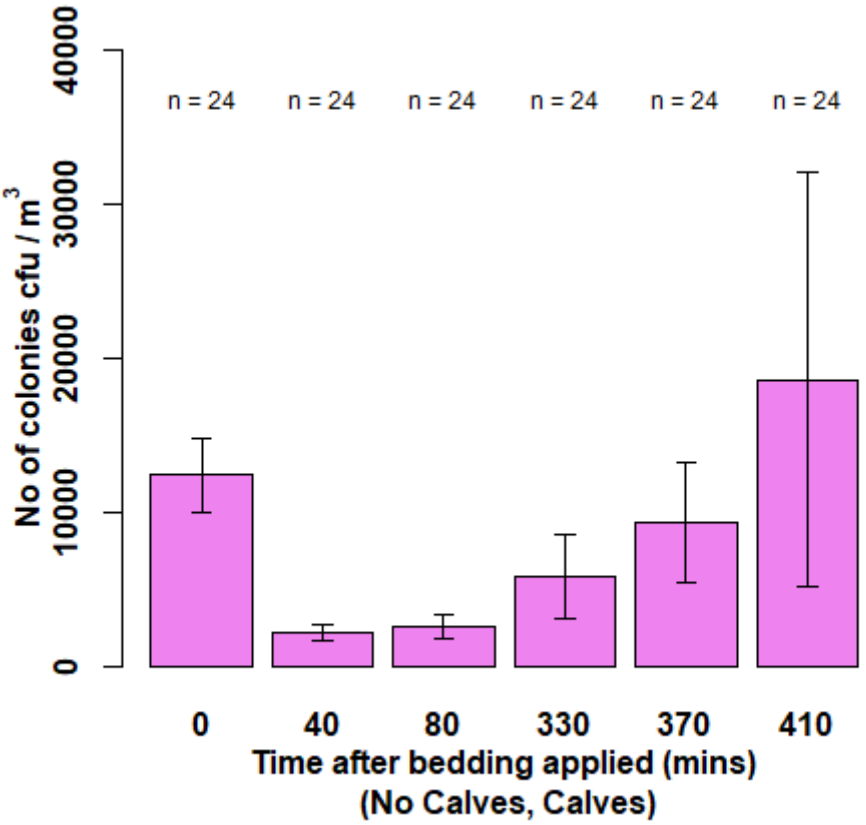

Figure 18 Effect of time after bedding applied (0, 40, 80, 330, 370, 410 mins) on total bacterial count (cfu/m3) when examining the presence of calves (n= number of observations)

5 Discussion

5.1 Thermal Imaging

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 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 & 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.

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 2oC which is similar to the present study.

The sensitivity and specificity analysis suggest that the model could not correctly identifying 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. 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.

Such further work would also be greatly enhanced by more academic collaboration. There will be a vast number of thermal images collected by the various studies which could be pooled and analysed in greater depth, perhaps by the use of machine learning techniques.

5.2 Behavioural response to air temperature & 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). 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.

Findings by Holmes & 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.

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(s) 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°C) as shown in the literature. If more 'extreme' air temperatures had been examined, for example -10°C and +25°C, then perhaps there might have been an effect of air temperature on the behavioural responses. A study by Ingram & 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°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 & 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.

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 et al. (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 & 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.

The results of this study have shown that calves show an aversion to increasing wind speed. The study has also provided evidence that suggests housing calves in an area that is free from draughts.

5.3 Calf performance & environment

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.

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). 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 & 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. 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).

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°C (mean entry age was 10 days). Every day after this, the LCT declined by 0.5°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.

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.

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. Future work could consider night and day differences in climate related variables and the effect 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.

5.4 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) who found that the measurements with no animals and no bedding were considerably lower than when animals and bedding was present.

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) due to its insulating properties. 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.

From this study it was 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.

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.

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. MacVean et al. (1986) mentioned that particles between 2.0 and 3.2µm had a significant effect on BRD. The results suggest (see Figure 15) 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.

These results have shown that the presence of calves affects the quality of air in the housing environment, 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.

6 Industry messages

The aim of this research was to investigate 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. Also the aim was to assess 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).

The key messages from this study are:

- Calves show an aversion to an increasing wind speed, so it is important to protect them from draughts
- The more time that calves are exposed to temperatures below their LCT at a very young age (within the first 6-14 days of life), the poorer the performance that can be achieved in terms of daily liveweight gain
- Calves provide another source of particles and bacteria into the housing environment, so think about stocking rates within calf housing
- Thermal Imaging cannot be reliably used in on-farm conditions to detect pyrexia in pre-weaned calves

From a review of the literature as part of this study, it has been apparent that there is very little research into calf housing (in particular some of the basic principles) and that there is a lot of variation in the advice available to farmers surrounding the subject of calf housing. This is a concern. Perhaps more attention should be given to this subject and an industry stakeholders group formed to ensure that everyone is 'singing from the same hymn sheet' as the calves of today are the herd of tomorrow.

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