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Novel approaches to improving calf health: new technology and data analytics



THE UNIVERSITY
of EDINBURGH

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Doctor of Philosophy,

College of Medicine and Veterinary Medicine,

The University of Edinburgh

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Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified. Parts of this work have been published in *Frontiers in Veterinary Science*, namely the body of chapters 2 and 4. I declare that permission from all authors was obtained for inclusion of the manuscript in this thesis.

Bethan Riley

31st of March 2025

Abstract

Calf disease significantly impacts both welfare and production. Bovine respiratory disease (BRD) and neonatal calf diarrhoea (NCD) commonly affect calves and are frequently missed by farm staff. Activity and milk feeding variables have been associated with BRD and NCD and are being investigated for use in disease detection tools. Saliva may also hold some potential for NCD detection.

Group housed calves had their activity and milk feeding variables recorded by a tri-axial accelerometer and automatic milk feeders and were scored for BRD in two trials. In the second trial, calves were scored for NCD and dehydration daily and had blood and saliva samples taken weekly and when they developed NCD.

Haematocrit was measured in all blood samples and electrolytes and serum proteins in a subset of thirty. All saliva samples had both pH and conductivity measured.

Four datasets were created. Healthy calves from the first trial were selected by excluding data three days either side of a diseased or intermediate BRD score or treatment event, giving a dataset of 31 calves with 10 days each. The association between intrinsic calf factors and activity and milk feeding variables was analysed. Calves with BRD were paired with a healthy calf on the same date, of the same sex, within seven days of age and ten kilograms of weight. Three days either side of peak disease were also taken for analysis. Mixed models analysed the association between BRD and activity and milk feeding variables.

The second trial's dataset was cleaned to remove the confounder of BRD and split into laboratory and behaviour variables. The dehydrated calves were matched with a healthy calf and a NCD and normally hydrated calf. The association between NCD with or without dehydration and saliva pH and conductivity, haematocrit and total protein were analysed using mixed models. Lin's correlation concordance was correlated between saliva pH and haematocrit in the larger dataset and saliva pH and strong ion difference in the smaller dataset. Mixed modelling was used to analyse the association of NCD with or without dehydration on milk feeding and activity variables.

Heavier healthy calves visited the milk more often for shorter visits and drank faster than their lighter counterparts. Older healthy calves had shorter lying bouts and were less active than younger calves. Summer born healthy calves lay down for longer, had more lying bouts and, more shorter standing bouts each day, than healthy calves born in autumn or winter. Healthy male calves drank more slowly, were less likely to consume their milk allowance and had longer lying bouts than healthy female calves.

Calves with BRD visited the milk feeder for longer for the three days following peak disease and drank more milk per feed on days -3 and -2 relative to peak disease. No other comparisons between diseased and healthy calves on any day were significant. Differences between these findings and those in the literature may be due to nutrition.

Saliva pH but not conductivity was associated with NCD, regardless of hydration status. Saliva pH was not associated with haematocrit or strong ion difference. Calves with NCD, regardless of hydration status were less active, less likely to consume their milk allowance and drank more slowly than healthy calves. Calves with NCD that were normally hydrated had fewer, longer lying bouts and visits to the milk feeder and more standing bouts than healthy calves. Dehydrated calves with NCD had longer visits to milk than either their normally hydrated diarrhoeic or their healthy counterparts.

In conclusion, young calves do show differences in behaviour when they are sick. Saliva pH is associated with diarrhoea so may have potential for a disease detection tool but the mechanism is unclear.

Lay summary

Young calves frequently become ill with diarrhoea or respiratory disease. This can severely affect their welfare as well as reducing growth and future production. In recent years technology has increasingly been used on farms including activity monitor and automatic milk feeders. These technologies may be able to help detect disease early, something that can be difficult particularly as labour is short on farms. With calves regularly visiting a teat it may also be possible to measure characteristics of the saliva to detect disease.

Calves were kept in groups and were health scored daily for respiratory disease over two trials. Their behaviour was recorded using automatic milk feeders and activity monitors. In the second trial calves were also scored for diarrhoea and dehydration daily and had saliva and blood samples taken weekly or after diarrhoea had developed.

The first set of data analysis aimed to find which calf factors were associated with healthy calf behaviour. Heavier healthy calves visited the milk more often for shorter visits and drank faster than their lighter counterparts. Older healthy calves had shorter lying bouts and were less active than younger calves. Summer born healthy calves lay down for longer, had more lying bouts and, more shorter standing bouts each day, than healthy calves born in autumn or winter. Healthy male calves drank more slowly, were less likely to consume their milk allowance and had longer lying bouts than healthy female calves.

Calves with respiratory visited the milk feeder for longer after they were ill and drank more milk per feed before they were ill.. There was no association between respiratory disease and a calf's activity. Differences between these finding and those in the literature may be as these calves were on a restricted milk ration.

Calves with or without dehydration had a lower saliva pH than healthy calves. However, saliva pH was not associated with blood measures of dehydration. Calves with diarrhoea with or without dehydration were less active, less likely to consume their milk allowance and drank more slowly than healthy calves. Calves with diarrhoea that were normally hydrated had fewer, longer lying bouts and visits to the milk feeder and more standing bouts than healthy calves. Dehydrated calves

with diarrhoea had longer visits to milk than either their normally hydrated diarrhoeic or their healthy counterparts.

In conclusion, young calves do show differences in behaviour when they are sick.

Saliva pH is associated with diarrhoea so may have potential for a disease detection tool but the mechanism is unclear.

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Dedication

This thesis is dedicated to my grandmother's Olive Riley and Bridie Brandreth and my aunt Una Brandreth. Three strong women who all valued education and supported me when I started this journey but sadly are not here to see me at its end.



Bridie Mary Brandreth
12th of November 1942 -
16th of May 2021



Una Brandreth
19th of September 1967 -
19th of March 2024



Christopher Alick Riley
9th of May 1932 -
6th of September 2010

Olive Elsa Riley
30th of August 1932 -
25th of August 2023

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B. Riley, M. Haskell, C-A. Duthie, A. Corbishley, C. Mason. 2023. The effect of neonatal calf diarrhoea with and without dehydration and calf level characteristics on blood parameters used to measure dehydration. *Cattle Practice*, 31 (1), 121. Poster presented at British Cattle Veterinary Association Congress 2023

B. Riley, C-A. Duthie, C. Mason, A. Corbishley, M. Haskell. 2022. 53. The association of neonatal calf diarrhoea with and without dehydration on milk feeding and activity variables in young pre-weaned artificially reared calves. *Animal - science proceedings*, 15 (1), 57-59. Presented at British Society Animal Science Conference 2024

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B. Riley. 2025, Feeding and activity behaviours in scouring calves with or without dehydration. *Cattle Practice*, 33 (1) In press. Presented at the British Cattle Veterinary Association Congress 2025

List of abbreviations

ADG	Average daily gain
BRD	Bovine respiratory disease
CUSUM	Cumulative sum statistical control chart
DDF	Denominator degrees of freedom
EMM	Estimated marginal means
GB	Great Britain
WCHS	Wisconsin calf health score
IL	Interleukin
NCD	Neonatal calf diarrhoea
NCD-H	Neonatal calf diarrhoea hydration status normal
NCD-D	Neonatal calf diarrhoea dehydrated
NDF	Numerator degrees of freedom
NSAID	Non-steroidal anti-inflammatory
PLF	Precision livestock farming
SAA	Serum amyloid A
SD	Standard deviation
SEM	Standard error of the mean
TNF- α	Tumour necrosis factor- α
TUS	Thoracic ultrasonography
UK	United Kingdom

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Chapter 1 Introduction and literature review

1.0 Preface

Prior to commencing on a project looking at novel approaches to calf health it is first important to understand the extent and nature of the problem at hand. This literature review will look at the literature describing issues surrounding calf disease and its diagnosis. It will then go on to describe the literature surrounding calf behaviour and its use to detect disease. The literature around technologies that measure variables other than behaviour to detect disease will be reviewed as will some of the human behaviour factors to consider when using technology to detect disease. Finally, the existing research on biomarkers in calves will be evaluated with a view to identifying a possible candidate for non-invasive testing.

1.1 Artificially reared calves

In 2024 1.48 million calves were registered to a dairy dam in the United Kingdom (UK) (Behera, 2025). Artificial rearing is a widespread practice for calves born on a dairy farm and calves are usually separated from their dam within a few hours of birth (Wagner *et al.*, 2013). Unlike on suckler beef farms where calves will remain with their dam drinking milk for around eight months artificially reared calves are typically only fed milk for two months (Machado and Ballou, 2022). On a typical dairy farm, some of the calves that are born will be dairy breed heifers that will be destined to enter the dairy herd themselves. These calves are usually reared by the enterprise of their birth or by another enterprise contracted to do so. They will then join the milking herd when they calve, which is targeted to be at twenty-four months of age. Traditionally, dairy bull calves would be euthanised due to their low value. After significant reputational challenges, in 2020 the UK dairy industry committed to the elimination of healthy calf euthanasia by 2023 (Agriculture and Horticulture Development Board, 2020). This has led to an increase in the use of female sexed dairy semen to breed heifers from the best cows. In the year leading up to April 2024 84% of the dairy breed semen sold was female sexed (Ruminant Health & Welfare Group, 2025). In turn, this has led to an increase in dairy cows bred to beef sires, with 52% of semen sold to dairy farms now being from a beef sire. The resulting dairy-beef cross-bred calves are usually sold to be reared and are then slaughtered as prime beef at twelve to thirty months of age. However, dairy cross beef heifers may occasionally be used for breeding in the suckler herd. The number of dairy cross beef animals has increased by 77% in Great Britain (GB) between 2013 and 2023 (Ruminant Health & Welfare Group, 2025). In Australia, the increase of dairy beef animals born into the dairy herd was a driver for ceasing the euthanasia of healthy calves (Vicic *et al.*, 2022). Despite considerable progress in this area recent research interviewing farmers operating block calving systems showed that dairy bull and dairy-beef calves are still perceived as having little value (Green *et al.*, 2025). The dairy-beef sector also allows us to reduce the greenhouse gas emissions of beef production, as the dam's emissions are assigned to her milk production (van Selm *et al.*, 2021).

Mortality is a significant challenge in calves. Twenty-five percent of on farm cattle deaths in GB occur before three months of age (Hyde *et al.*, 2020), but this figure does not entirely reflect the mortality of young animals on farm. Due to the way this data was collected, this excludes perinatal mortality. When this is explored further, mortality in dairy heifer calves less than three months of age is 4.96%, but this number is lower in beef calves (including beef cross dairy and purebred beef calves) at 2.86% (Hyde *et al.*, 2020). On French dairy farms in 2005, average mortality for dairy heifer calves was 5.38% and 5.78% for dairy beef cross calves (Raboisson *et al.*, 2013), A Chinese study indicated that mortality in Holstein heifer calves between three and sixty days of age is 5.5% (Zhang *et al.*, 2019). A Canadian study of dairy calves reported a mortality of 6% in pre-weaned heifers (Roche *et al.*, 2020). The sources of data in all these studies does not differentiate between artificially reared calves and calves suckling from a cow. Therefore, artificial rearing is assumed for the purebred dairy calves and dairy beef calves. The GB data does not differentiate between artificially reared dairy beef cross calves and pure-bred beef calves reared with their dam (Hyde *et al.*, 2020). On pasture-based New Zealand farms, mortality in artificially reared calves is approximately 4.1% between twenty-four hours of age and weaning (Cuttance *et al.*, 2017), Therefore, mortality appears broadly similar worldwide, but there is still much room for improvement. There is little information on the cost of mortality to the farm. Boulton *et al.* (2017) used data from 101 UK dairy farms to calculate the rearing cost of heifers, they estimated cost of rearing to be £1391.47 (£604.01 - £2842.96). They then assumed heifer mortality to be that found by previous studies (Brickell *et al.*, 2009, Brickell and Wathes, 2011), calculated the rearing cost of a heifer up to death and estimated that for each heifer that died £103.49 to £146.19 was added to the rearing costs of the remaining heifers depending on the number of heifers and the farms system. A similar study in the Netherlands estimated the rearing cost of a heifer to be €1520 on average and €47 to be the cost of mortality and culling per surviving heifer (Mohd Nor *et al.*, 2012). This is of particular concern as the median productive life of a cow in the UK is decreasing (Taylor-Holt *et al.*, 2025), this may affect the lifetime profitability of a cow. Considering the trade-offs between herd

replacement costs, calf opportunity costs, genetic opportunity costs and the cost associated with an aging cow a five-year productive lifespan has been suggested as a target (De Vries, 2020).

1.2 Is calf disease a problem?

Disease poses a significant welfare problem to calves and is costly to producers. The most frequent diseases in dairy calves are respiratory diseases and diarrhoea (Reiten *et al.*, 2018). In a study of eleven UK dairy farms, 48 per 100 calves (range 24 - 74) were diagnosed with neonatal calf diarrhoea (NCD) and 46 per 100 calves (range 20 - 78) were diagnosed with Bovine Respiratory Disease (BRD) in the first 9 weeks of life (Johnson *et al.*, 2017). A recent UK study across sixteen farms reported a slightly lower incidence of BRD at 38 per 100 calves in the first eight weeks of life but did not report the herd level incidence (Lindley *et al.*, 2025). In a Californian study of five herds, incidence of BRD was lower at 23 per 100 calves in the first nine weeks of life, with an estimated short-term cost of \$42.15 (£31.61) per case, but no measurement of NCD incidence was made (Dubrovsky *et al.*, 2018). A study of farms in Ontario and Minnesota found that NCD affected 23 per 100 calves in the first three months of life and BRD affected 22 per 100 calves in the first three months of life (Windeyer *et al.*, 2014). Another study in Ontario found that within-herd prevalence was 24% (range 8 - 46%) for NCD and 11% for BRD (range 0 – 60%) (Medrano-Galarza *et al.*, 2018). A cross-sectional study of 731 German dairy farms looked at disease prevalence and found that 18.5% of pre-weaned calves had NCD and 8.7% had BRD (Dachrodt *et al.*, 2021). Similarly, in an Irish study of spring calving herds, within herd prevalence ranged from 0% to 20%, with the overall prevalence being 4.7% (Donlon *et al.*, 2023c).

Disease is a significant welfare problem (Algers, 2004), as infections cause suffering (Mellor and Stafford, 2004). Both respiratory disease and gastrointestinal disease featured as highly ranked welfare consequences of all systems for pre-weaned calves by a panel of experts (EFSA Panel on Animal Health and Animal Welfare, 2023). The fatigue that may be associated with a disease process uses substantial resources and disease may also lower an animal's ability to access resources

(Algers, 2004). The inactivity associated with disease may also be considered evidence of a negative affective state (Fureix and Meagher, 2015). This is however difficult to confirm as testing is confounded by the reduced activity also caused by sickness (Lawrence *et al.*, 2025). Disease processes that lead to breathlessness or pain are particularly likely to cause suffering (Mellor and Stafford, 2004). This is why disease is important in both the third (health conditions) and fifth (mental state) domains of the Five Domains Model for assessing animal welfare (Mellor *et al.*, 2020). To assess the effect of disease on the welfare of calves. In recent years the concept of health-related quality of life has moved from human healthcare to be discussed in calves thus combining the domains of behaviour, mental state and health (Ramos *et al.*, 2025). This concept has been used to develop a scoring system for the detection of BRD (Bell *et al.*, 2023, Bell *et al.*, 2024).

In addition to being a welfare concern both BRD and NCD will increase the risk of mortality. Windeyer *et al.* (2014) found that 4.9% of NCD cases and 7.1% of BRD cases led to mortality. Mortality has also been estimated to be lower in some studies with Dubrovsky *et al.* (2019) reporting that 2.37% of BRD cases lead to mortality. Gulliksen *et al.* (2009) found that both NCD and BRD increased the risk of death at eight to thirty-one days of age, with hazard ratios of 2.94 and 6.49, respectively. Goh *et al.* (2024) that found that in calves that developed diarrhoea prior to sixty-six days of age, the hazard ratio of mortality in the subsequent twenty days was 2.48 and the hazard ratio of mortality 21 - 100 days after diarrhoea was 1.89. The results of the meta-analysis by Buczinski *et al.* (2021) showed a odds ratio of 2.85 for mortality in calves with BRD.

Disease in the pre-weaning period also influences growth. Neonatal calf diarrhoea, but not BRD, was associated with reduced growth in the first three months of life (Windeyer *et al.*, 2014). However, in a study of a single Mexican herd, both NCD and BRD were associated with reduced average daily gain (ADG) (Olivera *et al.*, 2025b). Johnson *et al.* (2018) found that the number of weeks with either BRD or NCD pre-weaning were associated with a reduction in ADG. In another study, treatment for BRD had a greater association with ADG than lung consolidation diagnosed by thoracic ultrasound (TUS) (Sáadatnia *et al.*, 2023). Jourquin *et al.*

(2023) found that pneumonia diagnosed by TUS was associated with reduced ADG and they also found that pneumonia that had lasted more than 30 days was associated with a greater effect on ADG. Holstein heifers that had BRD in the first sixty days of life were shown to be lighter at four hundred days of age than those that did not have BRD (Hurst *et al.*, 2021). In a study of over three hundred calves in one herd both the Wisconsin calf health score (WCHS) and lung consolidation on TUS were associated with a reduction in ADG (Cramer and Ollivett, 2019). A meta-analysis model incorporating data from ten studies concurred that BRD is associated with reduced ADG (Buczinski *et al.*, 2021). In a large American study one or more disease events, and the presence of the NCD pathogens, cryptosporidium or Giardia were associated with a reduced ADG (Shivley *et al.*, 2018). Differences between the effects seen may be due to different pathogens being predominant or differences in treatment efficacy.

Disease in early life may affect the long-term productivity of a dairy heifer. However, the evidence is mixed. Calves that experienced BRD at two to four months of age were less likely to calve by twenty-five months (odds ratio 0.6) than those that did not (Stanton *et al.*, 2012). Bovine respiratory disease in the first one hundred and twenty days of life is associated with an increase in the likelihood of leaving the herd prior to first calving and a reduction in first lactation milk yield (Schaffer *et al.*, 2016). Calves with extensive lung consolidation were more likely to leave the herd prior to first calving than those with no abnormality or milder disease, however no association with age at first calving was found (Adams and Buczinski, 2016). Closs and Dechow (2017) found that BRD in the first six months of life was associated with an increased risk of leaving the herd prior to first calving but not milk yield in the first lactation. Hurst *et al.* (2022) found that BRD at sixty to one hundred and twenty days of age was associated with reduced survival to first lactation, but BRD up to sixty days of age was not. There was no association between BRD and milk production (Hurst *et al.*, 2022). The detection of lung consolidation by TUS in the first fifty-six days of life has been associated with a decrease in first lactation milk yield but not age at first calving (Dunn *et al.*, 2018). In contrast, Teixeira *et al.* (2017) found no association between lung consolidation

at weaning and first lactation production but found an association with a reduction in reproductive performance. Bach (2011) found that there was an association between having four or more episodes of BRD and calving at an older age and reduced odds of surviving the first lactation.

The literature is not clear on whether NCD has a long-term effect on affected heifers. Heinrichs and Heinrichs (2011) found that days ill with NCD or cough in the first four months of life was negatively associated with 305-day mature equivalent milk, protein, and fat yields, but not age at leaving the herd or lifetime milk yield. In another study, diarrhoea pre-weaning was associated with an increase in the age at first calving and a 325 kg reduction in 305-day mature equivalent milk yield (Abuelo *et al.*, 2021). Aghakeshmiri *et al.* (2017) found that calves with NCD in the first month of life calved into the herd seven days later when compared to those that had not had NCD. Goh *et al.* (2024) found that calves that had NCD pre-weaning would calve four days older than their healthy counterparts, were more likely to die or be culled in the first lactation and had a higher peak somatic cell count. However, this was not supported by the findings of Mahendran *et al.* (2017) or Boccardo *et al.* (2019).

Unfortunately, there is limited information on the long-term productivity of artificially reared male calves or beef cross dairy calves. Schinwald *et al.* (2022) found that ADG was negatively associated with the proportion of days with an abnormal faecal score. A study in bull beef that did not differentiate between breeds estimated that both pleurisy and pulmonary consolidation at slaughter was associated with reduced estimated daily live-weight gain (Williams and Green, 2007).

In addition to the welfare concerns and costs incurred due to BRD, there is also a public health concern. Antibiotic resistance has been identified as one of the major challenges to human health in the twenty-first century (Conly and Johnston, 2005). Resistance has frequently been blamed on the livestock sector, and veterinary surgeons and farmers must accept some of the responsibility (Barkema *et al.*, 2015). Bovine respiratory disease is the leading reason for antimicrobial use in calves (Holstege *et al.*, 2018) and resistance has been observed in several of the

bacterial respiratory pathogens. A German study showed an increase in antibiotic resistance in both *Pasteurella multocida* and *Mannheimia haemolytica* between 2015 and 2020, with an increase in multidrug resistance also seen (Melchner *et al.*, 2021). A study in Canadian feedlots found that 21.3% of *P. multocida*, 2.4% of *M. haemolytica* and 3.4% of *Histophilus somni* isolates showed resistance to three or more antimicrobial classes (Rattanapanadda *et al.*, 2025). Multi-drug resistance was detected in 18% of *P. multocida* isolates from clinical cases in the UK in 2024 (UK-VARRS, 2025). Of current concern is that oral products for calves have increased by 110% since 2016 (UK- VARRS, 2025). Data from neonatal and pre-weaned calves in the UK showed that 37% and 34%, respectively of *Escherichia coli* isolates showed limited treatment options on antimicrobial sensitivity data in 2024 (UK-VARRS, 2025). This is like the 31% of *E. coli* isolates from the calf environment on Swiss dairy farms being multi-drug resistant (Bernier Gosselin *et al.*, 2025). This has led to increasing pressure for farmers and vets to reduce antimicrobial use. A target was set to reduce the mg/kg of antibiotics used on calf rearing units in GB by 25% between 2020 and 2024 (Responsible Use of Medines in Agriculture Alliance, 2020). However, progress against this target has not been assessed due to challenges in data capture (Responsible Use of Medines in Agriculture Alliance, 2024).

With the number of calves suffering mortality and the welfare and monetary cost associated with calf disease and mortality, this has become a priority for the industry in recent years. In a study where sixteen cattle welfare experts ranked welfare issues, neonatal morbidity and mortality was highly ranked as a priority welfare issue (Rioja-Lang *et al.*, 2020). This is not the case in all studies however, Bauman *et al.* (2016) found that calves were ranked below issues such as reproduction, udder health, and cost of disease when farmers, veterinarians and academic and government stakeholders were asked to rank management issues. However, animal welfare was the top priority for all stakeholders. A target was set to reduce calf mortality in the UK by 1% per year as part of a programme aimed at improving health and welfare (Responsible Use of Medines in Agriculture Alliance, 2020). Unfortunately, data availability meant that it was not possible to measure

progress against this target (Responsible Use of Medines in Agriculture Alliance, 2024).

1.3 Why do we need to improve disease diagnosis?

While calf health is vital, the literature suggests that clinical diagnosis of disease is poor on farm. Olson *et al.* (2019) found an incongruity between calves treated for disease in the first twenty-eight days of life by farm staff and those deemed to have abnormal clinical signs by an independent trained observer. Of the calves that the independent observers deemed to be showing signs of NCD or dehydration, only 6% received treatment from farm staff. When on-farm diagnosis is compared to subsequent post-mortem analysis of deceased animals, then there is only fair agreement between the two diagnoses (McConnel *et al.*, 2019). The meta-analysis by Timsit *et al.* (2016) showed that diagnosis of BRD in weaned cattle by stockmen in feedlots displayed a large range of sensitivity and specificity when compared with lesions detected at slaughter. Overall sensitivity was poor, and specificity was high. A UK study in calves twenty-one to sixty-one days of age showed that only 24.5% of calves classed as abnormal on TUS had been treated by the farmer (Baxter-Smith *et al.*, 2022). Clearly, improved diagnostic tools are needed.

Furthermore, early diagnosis allows targeted treatment and may reduce antimicrobial use (Belaid *et al.*, 2019). It is likely that monitoring needs to be frequent, as a study by Mahendran *et al.* (2017) showed that fifty-five calves died without having an abnormal WCHS when scoring was only carried out weekly. Baxter-Smith *et al.* (2022) found that 7.5% of calves classed as normal on TUS had been treated for BRD by the farm. Thus, tools need to be specific as well as sensitive.

1.4 Bovine respiratory disease

In this and following section, I will discuss the causative pathogens, environmental risk factors, and detection methods for BRD and NCD, respectively. Bovine respiratory disease may otherwise be known as pneumonia, and some farmers will call all respiratory disease pneumonia even if there is no involvement of the lungs.

1.4.1 Pathogens

Bovine respiratory disease is a multifactorial disease (Closs and Dechow, 2017) and is frequently seasonal (Denholm *et al.*, 2024, Murray *et al.*, 2016, Yamaguchi *et al.*, 2021). Bovine respiratory disease can be sub-divided into upper respiratory tract disease, clinical pneumonia, and subclinical pneumonia (Ollivett and Buczinski, 2016). Disease is often initiated by viruses before colonisation by bacteria (Workman *et al.*, 2019). While the different viruses are part of the same disease complex, they interact with the epithelium of the airway in different ways (Kirchhoff *et al.*, 2014). In a study of primarily pooled nasal swabs submitted to a laboratory from across the UK between 2020 and 2022, 77.6% of samples had at least two bacterial pathogens identified and 17.7% had two or more viral pathogens identified (Denholm *et al.*, 2024). The most frequent viral pathogen identified was bovine respiratory syncytial virus (21.6%) and the most frequent bacterial pathogen was *Pasteurella multocida* (93.4%). However, a Scottish study looking at diagnoses from samples taken at postmortem examination between 2014 and 2018 found a higher incidence of *Mycoplasma bovis* and *Mannheimia haemolytica* in calves submitted for postmortem examination (SRUC Veterinary Services, 2019). This may be as the later study looked at pre-weaned calves only, as *M. haemolytica* is less likely to be diagnosed in older calves (Denholm *et al.*, 2024). Increasing numbers of *M. Bovis* cases were identified by the UK passive surveillance network between 2012 and 2016, possibly due to increased sampling, as producer and clinician awareness of this pathogen increases (Animal and Plant Health Agency and Scottish Agriculture College Veterinary Services, 2017).

1.4.2 Risk factors

There is considerable evidence relating to the risk factors for BRD. Housing calves in groups that were stable throughout the pre-weaning period has been shown to reduce BRD incidence (Johnson *et al.*, 2021). Predisposing factors for BRD include failure of passive transfer of immunity (Windeyer *et al.*, 2014, Olivera *et al.*, 2025a), higher daily maximum temperature (Louie *et al.*, 2018), sharing air space with cattle up to eight months of age and a greater depth of wet bedding (Medrano-Galarza *et al.*, 2018). Dirty calves (> 25% of calf hide being contaminated with

faeces) are associated with increased rates of BRD when compared to clean calves (no manure seen on hide) (Calderón-Amor and Gallo, 2020). A systematic review showed that the evidence regarding associations between air pollutants (e.g. ammonia), temperature, relative humidity or bedding and BRD is not conclusive (Donlon *et al.*, 2023a, b). However, the evidence does suggest that increased air velocity is associated with BRD, but a consistent cut off has not been established (Donlon *et al.*, 2023a). A large study of eighty-three dairy farms from the same research group found that the air inlet design, milk feeding method, milk type, nesting score, mechanical ventilation, and area occupied by the calves were all associated with the WCHS (Donlon *et al.*, 2025). Season has been shown to be associated with clinical disease (Closs and Dechow, 2017), lung consolidation (Buczinski *et al.*, 2018a) and BRD found at necropsy (Murray *et al.*, 2016). A recent study found that different regions in California were affected by climatic conditions in different ways (Yamada *et al.*, 2025).

There is some evidence to suggest that increased levels of milk solids fed to calves decreases the risk of BRD (Johnson *et al.*, 2021). However, a systematic review found that thus far most of the evidence does not support this (Welk *et al.*, 2023). Stressors such as weaning and transportation will contribute to the severity of BRD (Griebel *et al.*, 2014). Evidence also suggests that risk factors are pathogen specific (Deepak *et al.*, 2021, Pardon *et al.*, 2020).

1.4.3 What is the gold standard for BRD diagnosis?

When assessing the suitability of a diagnostic test, a gold standard is required to compare to. A gold standard is the test with the highest sensitivity and specificity available (Berman *et al.*, 2020). The lack of a true gold standard for BRD diagnosis antemortem is an ongoing problem for research (Buczinski *et al.*, 2015). This is further hampered by a poor case definition for those needing treatment. While necropsy would be a suitable gold standard, the cost and invasiveness are prohibitive (Jones *et al.*, 1998). It is however considered extremely important for diagnosis of BRD in clinical settings (Mijares *et al.*, 2023). While lesions may be seen in the lungs at slaughter this will frequently be long after the disease event (Buczinski and Pardon, 2020). Some studies have simply assessed whether a

diagnostic test can predict mortality (Buczinski *et al.*, 2014) but as discussed in section 1.2 this only represent a small proportion of the calves that may be suffering from BRD. There are several possibilities for ante-mortem diagnosis of BRD, these include auscultation, disease scoring systems, and diagnostic imaging. Diagnostic imaging includes a range of modalities including thoracic ultrasound (TUS), radiography (x-ray), and computed tomography (CT). This section will discuss these main possibilities.

Auscultation would traditionally be used by the veterinary surgeon to diagnose respiratory disease, this is where a stethoscope is used to evaluate respiratory noise. However, when compared to TUS sensitivity was poor (Buczinski *et al.*, 2014). A recent study found inter-rater agreement was fair to good for most lung sound indicators (Princisval *et al.*, 2025).

Clinical scoring systems have been used increasingly in veterinary medicine and allow objective quantification of illness severity (Hayes *et al.*, 2010). There are two main clinical scoring systems for BRD in pre-weaned calves, the Wisconsin Calf Health Score (WCHS) and the Californian scoring system. The WCHS for BRD was first developed by McGuirk (2008) and has a scale of zero to three across five clinical signs. Love *et al.* (2014) developed the Californian scoring system for BRD in pre-weaned calves, which contains six clinical signs that are classified as either normal or abnormal. In a comparative study that compared both scoring systems to TUS and auscultation used in parallel, the sensitivity of using the scoring systems as a screening tool was 46% for the WCHS and 46.8% for the Californian system, and specificity was 97.4% and 91.2% respectively (Love *et al.*, 2016). Sensitivity was significantly improved when both scoring systems were used as a diagnostic tool for BRD in sick calves (Love *et al.*, 2016). The agreement between the two systems has been found to be excellent (Aly *et al.*, 2014). However, in a study of sixty calves comparing the WCHS to TUS, the WCHS had a sensitivity of 12% and a specificity of 98% (Hinnant *et al.*, 2024). One reason that has been suggested for a poor relationship between clinical scoring and diagnostic imaging is that there may be a lag between clinical disease and the development of imaging abnormalities (Fowler *et al.*, 2017).

A challenge in diagnosis is inter-observer agreement. When Buczinski *et al.* (2016a) examined the use of the WCHS, with relatively inexperienced observer's intra-observer agreement was only slight to fair. Berman *et al.* (2021) found that using a smaller scale of absence, mild/moderate and severe gave better repeatability between raters than the full –zero to three WCHS. The WCHS was suggested as a twice weekly screening tool by its creators (McGuirk and Peek, 2014). However, when used once weekly, there was no effect on calf health (Mahendran *et al.*, 2017). When used in a clinical setting, the cost of misclassification changes according to disease prevalence, thus the scoring system threshold for treatment needs to be lowered when prevalence increases (Buczinski *et al.*, 2018c). Interestingly, Lowie *et al.* (2024) found that a calf will display different clinical signs over the course of the day, this may mean that scoring needs to be conducted several times on the same day.

Thoracic CT has been used in research to image calves' lungs, however it has limited utility due to poor availability, high cost and the need for sedation (Berman *et al.*, 2020). Nonetheless it is the best ante-mortem test available (Berman *et al.*, 2020) and is highly correlated with necropsy results (Lubbers *et al.*, 2007).

Radiographic examination has been shown to be highly correlated with post-mortem examination of the lungs (Jones *et al.*, 1998). In another study CT and radiography had a moderate to high agreement (Fowler *et al.*, 2017). Improved imaging of the cranioventral lung can be achieved by restraining the calf with its leg pulled forward (Shimbo *et al.*, 2019).

The main diagnostic imaging modality used for BRD is TUS, which is used to detect consolidation of lung tissue. There is some evidence that consolidation may be associated with the involvement of bacteria on farms experiencing a respiratory epidemic (Oliveira *et al.*, 2020). However, like radiography this method can be time consuming, requires specialist equipment and training. Examination may be sped up by concentrating on the lung lobes where lesions are most likely to be present, namely the left and right cranial lobes and the middle lobe (Anteveli *et al.*, 2025). Alternatively, a quick scan method has been developed by the University of Ghent that consists of one movement on each side of the animal (Jourquin *et al.*, 2022).

Interobserver agreement varies slightly between studies. Buczinski *et al.* (2013) found it to be moderate to excellent depending on experience when examining live calves, in contrast the same group found it to be moderate when examining video footage of TUS (Buczinski *et al.*, 2018b). A recent UK study found fair agreement when using a 6-point score (as described by Ollivett and Buczinski (2016)) however agreement varied between raters and ultrasound technique (Lindley *et al.*, 2024). An advantage of TUS is that it can be combined with a systematic clinical scoring system (e.g. WCHS) to sub-type BRD into pneumonia (positive clinical score and TUS), sub-clinical pneumonia (positive TUS and negative clinical score) and upper respiratory tract disease (positive clinical score and negative TUS) (Ollivett and Buczinski, 2016). This may allow more targeted treatment and judicious use of antibiotics (Lindley *et al.*, 2025). A disadvantage of TUS is that lesions that do not extend to the lung surface will not be identified as ultrasound cannot penetrate the tissue/air interface (Babkine and Blond, 2009). Compared to other diagnostic imaging modalities TUS has the advantage of being more portable and has no radiation concerns.

A study of sixty-two calves found that TUS was better correlated with post-mortem examination findings than a modified version of the WCHS (Porter *et al.*, 2021). When compared to post-mortem findings in euthanized calves with no clinical BRD (as determined by WCHS), the sensitivity of TUS for detecting lung lesions was 94% and specificity 100%, but the study was small, with only twenty-five calves included (Ollivett *et al.*, 2015). Interestingly, Cramer and Ollivett (2019) found that both WCHS and TUS were associated with ADG, however these effects were separate and not interactive. An Irish study showed a weak correlation between WCHS and TUS scores, however only TUS scores were associated with ADG (Rhodes *et al.*, 2021). Severe lesions detected using TUS were associated with a reduction in ADG of 0.12 kg /per day pre-weaning, while having a WCHS above the clinical threshold (≥ 5) was not associated with ADG (Cuevas-Gomez *et al.*, 2021). Interestingly, Lowie *et al.* (2022) found that TUS was only associated with spontaneous cough when comparing it to both the WCHS and the California system, and that using cough alone had a sensitivity of 37.4% and specificity of 85.7% when compared to TUS.

Interestingly, Masset *et al.* (2022) who found almost perfect agreement between TUS examination of the entire thorax and the cranial part alone. This gives credibility to the suggestion that diagnostic accuracy may be reduced in older calves as it becomes more difficult to access the first and second intercostal spaces to visualise the cranial lung lobes (Ollivett *et al.*, 2015).

Care should be taken in interpretation of some of these studies. For instance, Buczinski *et al.* (2014) gave sensitivity and specificity of TUS for mortality despite only four out of one hundred and six calves dying within the study period. Thus, bias may be introduced by small sample sizes. Another factor to consider is that both TUS and clinical scoring will be influenced by the pathogen responsible for disease (Baruch *et al.*, 2019). There is some suggestion that consolidation depth may be associated with the presence of a bacterial infection (Clinquart *et al.*, 2025). Bronchoalveolar lavage (BAL) samples the cells within the airways, but is considered an invasive technique (Ollivett *et al.*, 2015). A cut-point for a differential cell count of the airways may be used to diagnose the presence of disease. When compared to the presence of lesions on the necropsy of euthanized calves BAL has been shown to have a sensitivity of 81% and specificity of 75% for sub-clinical BRD when using a cut off of $\geq 4\%$ neutrophils (Ollivett *et al.*, 2015). However, in another study BAL differential cell count corresponded poorly with TUS and WCHS (Van Leenen *et al.*, 2020). It was suggested that this may be due to the role of allergy and inflammation in BRD rather than pathogens being the primary instigator (Van Leenen *et al.*, 2020). Some studies such as Schaeffer *et al.* (2012) use a combination of methodologies as the gold-standard including haematology results.

Several authors have used Bayesian techniques to compare diagnostic methods. Bayesian techniques can be used when no test can be considered perfect. Boccardo *et al.* (2023) gave standardised descriptions for sounds heard on auscultation. Including increased breath sounds in the criteria lead to poor specificity. However, when increased breath sounds was removed from the criteria specificity was 90.8% and sensitivity was 81.7% (Boccardo *et al.*, 2023). This performance was better than that calculated by Buczinski *et al.* (2016b) who found that auscultation has a sensitivity of 72.9% and a specificity of 53.3%. Thoracic ultrasonography gave

greater diagnostic accuracy than auscultation in that study (sensitivity 76.5%, specificity 92.9%) (Buczinski *et al.*, 2016b). Donlon *et al.* (2024) compared different interpretations of the WCHS to TUS using Bayesian latent class analysis they found that specificity varied from 94% to 98% and sensitivity from 34% to 58%. The Californian score was comparable with a specificity of 91% and sensitivity of 56% (Donlon *et al.*, 2024). Berman *et al.* (2019) found that increasing the depth of consolidation cut off when interpreting TUS improved specificity with minimal impact on sensitivity. Interestingly, contrary to the findings of Ollivett *et al.* (2015) and Masset *et al.* (2022) discussed above greater accuracy was achieved by using the threshold of ≥ 3 cm consolidation caudal to the heart (sensitivity: 89%, specificity: 95%) (Berman *et al.*, 2019). No difference between the diagnostic accuracy of TUS and thoracic radiography was found when compared to thoracic CT using a two-stage Bayesian method (Berman *et al.*, 2020). When comparing TUS and the WCHS to each other using a Bayesian framework, TUS (sensitivity: 79%, specificity: 94%) performed better than the WCHS (sensitivity: 62%, specificity: 74%) (Buczinski *et al.*, 2015).

1.5 Neonatal calf diarrhoea

Neonatal calf diarrhoea is otherwise known as neonatal enteritis and is colloquially known as scour. Neonatal calf diarrhoea may be infectious in origin but may also be nutritional or due to rumen drinking.

1.5.1 Pathogens

Several pathogens may be responsible for NCD. These include bacterial, viral and protozoal pathogens (Heller and Chigerwe, 2018). The protozoal parasite cryptosporidium was the most frequently identified NCD pathogen in UK diagnostic samples between 2011 and 2016 (Animal and Plant Health Agency and Scottish Agriculture College Veterinary Services, 2017). The number of cryptosporidium cases increased year on year between 2011 and 2015 (Animal and Plant Health Agency and Scottish Agriculture College Veterinary Services, 2017). Rotavirus was the second most common pathogen, but it was identified less frequently year on year (Animal and Plant Health Agency and Scottish Agriculture College Veterinary

Services, 2017). This is consistent with cryptosporidium and rotavirus being the most frequent causes of death identified in a Scottish study looking at reasons for mortality in pre-weaned dairy calves submitted for postmortem examination between 2014 and 2018 (SRUC Veterinary Services, 2019). Bacterial enteric pathogens include *E. coli*, *Salmonella* species (Barkley *et al.*, 2021). Calves infected with *Salmonella* spp. are more likely to die than those infected by another pathogen (Barkley *et al.*, 2021). Coccidiosis is another protozoal parasite and is more frequently found in older calves (Animal and Plant Health Agency and Scottish Agriculture College Veterinary Services, 2017).

1.5.2 Risk factors

A variety of factors have been associated with a reduced incidence of NCD, including feeding probiotics, providing supplemental vitamin E and selenium at birth and the application of fresh bedding every two to three days, rather than every seven or more days (Medrano-Galarza *et al.*, 2018). The benefit of feeding probiotics is not surprising, as Scully *et al.* (2024) found that the microbial diversity in calf faeces was lower when diarrhoea started than it was at day seven of age, however no difference between the healthy calves and those that went on to develop diarrhoea at seven days of age. Oikonomou *et al.* (2013) found that the microbiota profiles were different between calves that did and did not suffer from NCD in the first four weeks of life. The feeding of waste milk containing antibiotics increases the risk of NCD (Penati *et al.*, 2021). Dry bedding has been associated with lower faecal scores (Bonizzi *et al.*, 2022). Risk factors for NCD include cleanliness of the calving area (Klein-Jobstl *et al.*, 2014), sharing the air space with animals more than nine months of age (Medrano-Galarza *et al.*, 2018), increased levels of milk replacer (Scoley *et al.*, 2019) and dirty calves (> 25% of calf hide being contaminated with faeces) (Calderón-Amor and Gallo, 2020). Interestingly, Glover *et al.* (2019) found that birth weight is not associated with mortality and was only associated with faecal consistency scores in one out of the three farms studied. However, Carrol *et al.* (2022) found that a low birthweight was associated with more treatments prior to weaning.

Nutritional scours is the term colloquially used for diarrhoea that is believed to be related to the calf's diet. This may include improper mixing of milk replacer or improper handling of milk or milk replacer (Heller and Chigerwe, 2018). A higher level of milk replacer has been associated with higher faecal scores (indicative of NCD) (Olagunju *et al.*, 2024, Quigley *et al.*, 2006, Brown *et al.*, 2023), but not increased mortality (Quigley *et al.*, 2006). However, in a small study there was no difference in either faecal scores or NCD treatment rates between groups fed different volumes and concentrations of milk (Kmicikewycz *et al.*, 2013). A larger study concurred with this (Chapman *et al.*, 2016). The composition of the milk replacer appears to play a role in nutritional diarrhoea. Supplementation of milk replacer with additional fatty acids has been shown to lower faecal scores (Esselburn *et al.*, 2013). Calves fed a whole-milk based milk replacer were more likely to require NCD treatment than those fed high lactose or high fat milk replacer (Wilms *et al.*, 2022). There has been some suggestion that housing and cleanliness have a far greater impact on diarrhoea than elevated levels of milk replacer (Khan *et al.*, 2011). A higher level of calf starter is not associated with an increase in faecal scores or treatments for NCD (Nejad *et al.*, 2013).

Rumen drinking is a significant cause of preweaning mortality (Animal and Plant Health Agency and Scottish Agriculture College Veterinary Services, 2017). Rumen drinking is more likely in calves drinking from an open bucket and in calves fed a low level of milk (Animal and Plant Health Agency and Scottish Agriculture College Veterinary Services, 2017).

1.5.3 Diagnosis

Diagnosis of NCD is usually by assessment of the faeces (Pempek *et al.*, 2019). In clinical cases, physical examination is a better predictor of outcome than serum biochemistry (Trefz *et al.*, 2017). For research use, there is a variety of metrics used to define NCD, with studies using one to eight metrics (Wilson *et al.*, 2023). Faecal consistency alone is the most frequently used metric, however there is a lack of uniformity in definitions across studies, which makes comparing studies challenging (Wilson *et al.*, 2023).

1.6 Calf Behaviour

Calf behaviour has long been known to change when a calf is sick. This section will discuss the changes in behaviour seen in disease, other factors that affect calf behaviour and technologies used to detect and record behaviours.

1.6.1 Behaviour changes in disease

Sickness behaviour is the term used for a variety of non-specific symptoms seen in illness, these symptoms may include anorexia, listlessness, reduced social exploration, weakness, hypersomnia, and malaise (Kent *et al.*, 1992), and is part of the body's sickness response. The sickness response has three aspects fever, sickness behaviour, and activation of the hypothalamic pituitary adrenal axis (Dantzer, 2004). The brain coordinates three aspects of the sickness response in response to cytokine production (Dantzer, 2004). Cytokines are inflammatory mediators produced by the innate immune system (Dantzer, 2004). The current hypothesized mechanism is that sensory neurons are stimulated directly or indirectly by cytokines at the site of inflammation (Dantzer, 2023). Thus, a signal is conducted via the central nervous system to the brain, leading to an increase in sensitivity to cytokines along the relevant neural pathway (Dantzer, 2023). Pathogen-associated molecular patterns in the general circulation stimulate cytokine production from the macrophages in the brain's ventricles which in turn stimulate more cytokine production by the microglial cells in the brain parenchyma (Johnson, 2002). Currently, we do not understand what happens once the immune signal has reached the brain although it appears that prostaglandin plays a role (Dantzer, 2023).

A fever is a crucial element of the bodies defence against disease as it reduces the ability of pathogens to replicate and potentiates some immunological responses (Hart, 1988). The metabolic demands of a fever cannot be sustained without changes in behaviour to reduce energy demands for other functions (Millman, 2007). Thus, changes in behaviour are seen to reduce other energy demands, reduce heat loss, and increase heat production. However, sickness behaviour and fever can occur separately (Kent *et al.*, 1992).

Sickness behaviours are therefore an adaptive strategy to combat disease by providing suitable conditions for recovery and reducing the spread of disease (Hart, 1988). There is however some discussion over whether the sickness response is only adaptive at “optimal levels of sickness,” beyond which the sickness becomes pathological (Sylvia and Demas, 2017). Sickness behaviours appear to be controlled at least in part by the brainstem (Ilanges et al., 2022). The presence of disease changes the motivation of the animal to conduct different behaviours (Johnson, 2002). Some sickness behaviours are well recognised by clinicians and farmers as clinical signs of disease (Millman, 2007).

The degree to which an animal displays sickness behaviours may affect its prognosis. In rabbits an increase in sleep following infection is seen and rabbits that die from their infection had slept less than those that did not (Toth *et al.*, 1993). In addition to the physiological mechanisms regulating sickness behaviours both environmental and social factors may influence their expression (Sylvia and Demas, 2017). Thus, providing suitable conditions for a calf to display sickness behaviours may be important in maintaining its welfare and aid in detecting disease.

Several studies have explored how calves' responses to interactions with humans may change in disease. Calves are more likely to approach a novel object or stationary human when not displaying signs of BRD or fever, this suggests that fever or BRD reduces exploratory behaviour (Cramer and Stanton, 2015). This finding was incorporated into the development of a behavioural scoring system which also incorporated isolation from the group, lethargy, willingness to rise and posture (Cramer *et al.*, 2016). However, this system had a sensitivity of only 48.4% for severe BRD with a specificity of 79.4% (Cramer *et al.*, 2016). An attitude score system developed by the same group also showed poor (23%) sensitivity for BRD (Cramer *et al.*, 2019).

1.6.1.1 Standing, lying, and walking behaviours

Standing and lying behaviours change in sick calves, however, these changes in behaviour are not consistent between studies. Following an injection of lipopolysaccharide calves lay down for longer and had more longer bouts of standing but being inactive (Borderas *et al.*, 2008). Both Hanzlicek *et al.* (2010) and

Hixson *et al.* (2018) induced BRD using endoscopic inoculation of *M. haemolytica*. Hixson *et al.* (2018) found that lying time was longer on the day of inoculation and Hanzlicek *et al.* (2010) found that the percentage of time standing was decreased between days two and six after inoculation. Calves took fewer steps after inoculation compared to before inoculation (Hanzlicek *et al.*, 2010). Swartz *et al.* (2017) found that calves also took fewer steps in naturally occurring BRD. In a study of older weaned calves, reduced activity was shown to be associated with BRD (Tomczak *et al.*, 2019). White *et al.* (2012) found that following inoculation of *M. bovis* weaned calves displaying a greater level of illness travelled less distance than those with milder symptoms. Duthie *et al.* (2021) found that calves with naturally occurring BRD had longer lying bouts for the two days prior to the day of peak illness and a longer total lying time on the day prior to peak illness. Calves with BRD lay down for longer each day in fewer bouts and were less active than their healthy counterparts (Cantor and Costa, 2022). In contrast, Swartz *et al.* (2017) found no changes in total lying time, but a reduction in the number of lying bouts from two days prior to three days after diagnosis. The reduction in activity may be due to a reduction in play behaviour in sick calves (Vázquez-Diosdado *et al.*, 2024). These differences between studies may be due to the differences in methodologies and technologies used.

Changes in lying behaviour have also been shown in NCD. Lowe *et al.* (2019b) found that prior to the appearance of clinical signs of NCD, the duration of lying bouts increases, with a decrease in lying bout frequency. In contrast, Sutherland *et al.* (2018) only found a tendency for reduced lying bout frequency and for reduced lying time on the day of diagnosis in calves with NCD. Studds *et al.* (2018) also found a tendency for a decrease in lying time in calves with NCD. Goharshahi *et al.* (2021) found that increased lying times were associated with NCD developing on the subsequent day. Furthermore, the amount of time spent both active and inactive on the day prior to disease was also associated with the odds of disease development. Interestingly, Swartz *et al.* (2020) found an increase in the number of lying bouts from day seven to day three prior to diagnosis of NCD and a decrease in lying bout duration from day six to day three prior to diagnosis. Some studies do

not differentiate between different conditions in their analysis. Belaid *et al.* (2020) found that sick veal calves had a reduced number of lying bouts between 2 days prior to diagnosis and nine days after diagnosis, sick calves also took fewer steps on the day prior to and day of diagnosis. While studies differ in their outcomes, overall changes that can be associated with lethargy are seen in calves with disease. The exact behavioural changes may be affected by several factors including pathogen, nutritional status and social environment as discussed below.

1.6.1.2 Feeding behaviours

Feeding behaviour is also known to change in sick calves. Visits to a calf feeder may be defined as rewarded (i.e. milk is consumed) or unrewarded (i.e. no milk is consumed). Svensson and Jensen (2007) found an association between unrewarded visits to the feeder and the presence of NCD or BRD, but no examination of behaviour changes prior to disease was made. In another study calves with BRD drink 1.6 L less milk on the day of disease diagnosis than their healthy counterparts (Swartz *et al.*, 2017). Cantor and Costa (2022) also found that calves with BRD drank less milk, however there was no interaction between BRD status and day relative to peak disease. Another study found that calves visited the feeder less often on days -3 and -1 to 3 relative to peak disease, consumed less milk on the day prior and two days after peak disease and spent less time at the feeder on the day of peak disease (Duthie *et al.*, 2021). Conboy *et al.* (2021) found that diseased calves drank a smaller proportion of their daily allowance and drank more slowly. In a challenge model, it was shown that calves infected with *M. haemolytica* had reduced milk feeder visits, but there was a significant time by treatment interaction with behaviours returning to normal within a few days (Hixson *et al.*, 2018). Johnston *et al.* (2016b) found that unrewarded visits to the milk feeder decreased in the three days prior to BRD diagnosis, but there was no reduction in milk intake. However, Knauer *et al.* (2017) found that unrewarded visits to the feeder only reduced on the day of BRD diagnosis, with a reduction in drinking speed seen on the same day. Interestingly, calves that go on to relapse drink less milk more slowly and have fewer unrewarded visits after treatment than their counterparts who go on to

recover (Cantor *et al.*, 2022b). All this research was conducted on calves in a restricted feeding system.

Changes in feeding behaviours are also seen in calves with NCD. Lowe *et al.* (2019b) found that milk consumption decreased prior to clinical signs of NCD. Sutherland *et al.* (2018) concurred with this finding. However, Katsoulos *et al.* (2020) found that milk consumption did not significantly reduce in mild cases of NCD (treated with oral fluids) but did in those requiring more intensive treatment. In contrast, calves with NCD drank more slowly from four days prior to diagnosis and had a reduction in unrewarded visits from two days prior to diagnosis (Knauer *et al.*, 2017).

Sutherland *et al.* (2018) also found a reduction in unrewarded visits in the 2 days prior to NCD diagnosis. Conboy *et al.* (2021) found that calves with NCD drank less of their daily allocation and drank more slowly than their healthy counterparts. In a study that did not differentiate between BRD and NCD diseased calves drank less milk from five days prior to farmer diagnosis until three days after diagnosis (Morrison *et al.*, 2022). They also had less unrewarded visits from three days prior to diagnosis until two days after diagnosis and drank more slowly from four days prior to diagnosis until the day after diagnosis. All this work was conducted in restricted fed calves. Changes in non-nutritive visits to the feeder are thought to be indicative of a reduction of sampling behaviour, where animals attempt to learn more regarding their surroundings (Weary *et al.*, 2009). As previously highlighted by Morrison *et al.* (2021) there is a lack of consistency in both the reporting of study designs and disease outcomes used, making direct comparisons challenging.

Very few studies consider behaviours surrounding solid feed intake in sick pre-weaned calves. Belaid *et al.* (2020) found that sick calves spent less time at the feed bunk prior to disease diagnosis with a significant difference seen from ten days prior to diagnosis. The number of visits to the feed bunk were also lower on the day prior to and the day of diagnosis. Hixson *et al.* (2018) found a reduction in the time spent eating concentrates in younger pre-weaned calves, but not older pre-weaned calves. Another area with limited information is water consumption. Lowe *et al.* (2019b) found that the duration of visits to the water trough increased in the days prior to diagnosis of NCD. Wenge *et al.* (2014) found that water intake increased in

calves with NCD. Both solid feed and water consumption warrant further work, especially as dehydration is a significant cause of mortality in calves with NCD (Hulbert and Moisa, 2016). Overall calves consume less nutrition, drink their milk more slowly and show fewer sampling behaviours when suffering from disease.

1.6.1.3 Other considerations when analysing the association between disease and behaviour.

A further consideration is that the behavioural changes seen with disease may be affected by the calves' milk allocation. Following the identification of illness, milk intake and frequency of visits will decrease, and the duration of visits increase where calves are fed an elevated level of milk/milk replacer. However, when calves are fed a low level of milk/milk replacer, the duration of visits decrease and no difference in visit frequency or milk intake is seen (Borderas *et al.*, 2009). In contrast, Lowe *et al.* (2021) found that only the number of visits to the feeder increased in sick calves with an allowance of five litres of whole milk daily. In calves that had access to ten litres daily, the total number of visits also increased, as did the percentage of visits that were rewarded, and the percentage of allowance consumed decreased.

Intrinsic calf factors such as sex may affect the way animals respond to a disease challenge. In Brahman cattle sex has been shown to influence the sickness response with bulls displaying a greater level of sickness behaviours than heifers (Carroll *et al.*, 2015). This was particularly interesting as heifers has a greater and more sustained febrile response to the lipopolysaccharide challenge. A calf's personality may influence its response to disease. Calves that displayed highly fearful traits had a greater change in milk intake and drinking speed following diarrhoea diagnosis than less fearful calves (Woodrum Setser *et al.*, 2024). In contrast, calm bulls displayed more sickness behaviour than their fearful counterparts following challenge with the endotoxin lipopolysaccharide (Burdick *et al.*, 2011).

Another consideration is the dose of infectious agent or severity of disease. Ballou *et al.* (2011) found that only calves that received higher doses of intravenous *E. coli* displayed sickness behaviours. The work of Szyszka and Kyriazakis (2013) on lungworm showed that the level of infection will affect the sickness behaviour

displayed. Cramer *et al.* (2020) found that calves with clinical pneumonia (i.e. TUS changes and positive WCHS) drank more slowly than those with sub-clinical pneumonia (only TUS changes) or those with no BRD, however there were no differences in milk intake, average meal size, rewarded visits or unrewarded visits. This is supported by the findings of Toaff-Rosenstein *et al.* (2016) that the degree of sickness behaviour seen in BRD was related to the gross pathology of the lungs.

1.6.2 Other factors that are associated with calf behaviour

When analysing changes in behaviour with disease it is important to remember that a variety of environmental and management factors can also affect behaviour including flooring, pen size, age, and the presence of other calves (Bonk *et al.*, 2013). Furthermore, there is considerable diurnal variation in calf standing behaviour (Hill *et al.*, 2013).

For instance, the age of the calf has been shown to have a major effect on behaviour. Calves had more lying bouts and less time at the feeder at forty days of age than at twenty-six days of age (Jensen *et al.*, 2020). Standing time has been shown to increase by 0.52 minutes per day of age (Hill *et al.*, 2013). Older calves have been shown to drink more milk, quicker than younger calves (Montes *et al.*, 2023) and spend less time running (Sutherland *et al.*, 2014).

Calves are more likely to play for less time than average as they get older (Reipurth *et al.*, 2020). The total daily time spent playing and the number of instances of play have been shown to be lower in older pre-weaned calves (Vázquez-Diosdado *et al.*, 2024). Once step down weaning commences and after weaning total daily time spent playing and the number of instances of play both increase (Vázquez-Diosdado *et al.*, 2024). Age has also been shown to influence behaviour during transport of calves less than ten days old (Jongman and Butler, 2014). Some studies have shown interactions between age, housing, and sex. Group housed female calves and pair housed calves became less active with age but male group housed calves and individually housed calves did not (Thorup *et al.*, 2021).

Breed and sex can also influence calf behaviours, this may influence the utility of behaviour-based disease detection tools in different demographics of calves. Both

breed and sex are associated with flight speed and behaviour in the handling chute (Hoppe *et al.*, 2010). Breed may affect the emotional state of dairy calves, however herd may be a confounding factor (Brscic *et al.*, 2019). Holstein calves were shown to be more fearful than Holstein cross or Jersey cross calves (Calderon-Amor *et al.*, 2020). In another study, male Jersey calves appeared more curious and less fearful than male Friesian cross Angus calves (Lauber *et al.*, 2009). Female calves have been shown to display more avoidance behaviours than their male counterparts (Ellingsen-Dalskau *et al.*, 2020). Holstein-Friesian female calves spend more time walking than their male counterparts but no differences in lying or standing time (Ugwu *et al.*, 2021). Group-housed female calves under 44 days of age were more active than their male counterparts (Thorup *et al.*, 2021).

A calf's social environment will influence its behaviour. Pair housed calves consume their milk in more frequent smaller meals and consumed more concentrate than their individually housed counterparts (Miller-Cushon and DeVries, 2016). Calves housed in groups of four or eight calves spent more time walking and standing and less time lying than pair housed calves (Abdelfattah *et al.*, 2013). They also spent less time manipulating objects and self-licking (Abdelfattah *et al.*, 2013). Calves housed in groups spent more time exploring and less time standing than those housed individually (Lv *et al.*, 2021). Calves pair housed from birth spent more time lying socially when grouped at approximately thirteen days of age than those individually housed from birth, however no difference in social play was seen (Lindner *et al.*, 2021). Similarly, Gingerich *et al.* (2023) found that calves paired in the first two weeks of life and then moved into group pens were bolder during open field and novel object tests and approached an unfamiliar calf less than calves that were individually housed prior to grouping. Locomotor play was also higher in pair housed calves when compared to individually housed calves (Valníčková *et al.*, 2015, Zhang *et al.*, 2021). This is not consistent with the finding of Jensen *et al.* (2015) that calves housed individually engage in more locomotor play but not more play overall when compared to pair housed calves. This is as pair housed calves can also engage in social play. In another study group-housed calves were more active than pair housed calves, which in turn were more active than individually housed

calves (Thorup *et al.*, 2021). However, McKay *et al.* (2025) found that pair and group housed calves played more than individually housed calves but there was no difference between the pair and group housed calves. It has been suggested that individually housed calves have less opportunity to express active behaviours as when placed in a novel arena test, they are more active than pair housed calves (Bučková *et al.*, 2021, De Paula Vieira *et al.*, 2012). Group housed calves were shown to eat more concentrate and play more in a recent meta-analysis (Donadio *et al.*, 2025). They also eat more of a novel concentrate feed (Whalin *et al.*, 2018). Evidence also suggests that cognitive development is impaired in individually housed calves when compared to their pair housed counterparts (Gaillard *et al.*, 2014, Meagher *et al.*, 2015). A menace response is a blink when a visual threat is applied to the eye without touching it and measures the visual pathway but is considered a learnt response. Group housed calves developed a menace response at a younger age than their individually housed counterparts, possibly due to there being more visual threats to the eyes in group-housing (Raofi *et al.*, 2009). The age at grouping may influence these behaviours, calves that were group housed at three days of age had more social interaction and performed more play behaviours than those grouped at seven or fourteen days of age (Abdelfattah *et al.*, 2018). Social housing also influences a calf's response to weaning and other stressful events. Pair housed calves consumed more calf starter at a greater rate during weaning than individually housed calves (Overvest *et al.*, 2018). When moved into groups after weaning, calves previously housed in pairs lay down for longer than those that had been housed individually (Knauer *et al.*, 2021, Liu *et al.*, 2019). Compared to individually housed calves previously pair housed calves eat more concentrate, more frequently and begin eating concentrate sooner when grouped after weaning (De Paula Vieira *et al.*, 2010). When previously individually housed weaned calves are introduced to a group at pasture their behaviour is significantly different to those already at pasture. However, these differences decrease over time (Horvath and Miller-Cushon, 2018).

Maternal factors may also influence the calf. The pre-natal environment may influence factors such as birth weight, dystocia, immune function, and calf survival

(Perry *et al.*, 2019). Calves with a greater birth weight or born to an older dam have a greater drinking speed and drink more milk when compared to a calf with a lower birth weight or born to a younger dam (Montes *et al.*, 2023). Calf lying behaviour may be influenced by maternal management during the dry period, with calves born to cows kept at pasture during the dry period having longer lying periods than calves born to cows kept indoors but exercised daily (Black *et al.*, 2017). Calf social behaviour has been shown to change with the dam's stocking density during the dry period (Fujiwara *et al.*, 2020).

Several factors around feeding methods and milk allowance may also influence calf behaviour, for example the number of calves per teat. Jensen (2004) found a greater level of competition with more displacements, a higher drinking speed and less time at the feeder when there were twenty-four calves to a teat compared to when there were twelve. The nutritional composition of the milk replacer will influence milk feeding behaviours (Wilms *et al.*, 2024). Calves fed 10% of their body weight had twelve times the number of unrewarded visits, spent twice as much time on the teat and stood for longer than calves fed *ad libitum* (De Paula Vieira *et al.*, 2008). In another study calves fed six litres of milk per day had more pauses and performed more sucks during each feeding bout but only tended to spend longer at the teat compared to those fed *ad libitum* (Todd *et al.*, 2018). However, no effect on time spent lying or active was found. Not all studies concur, Dennis *et al.* (2018) found no difference in the daily duration of rumination, eating (milk replacer and starter) or activity was found according to milk replacer regime. Krachun *et al.* (2010) found that calves on a lower milk intake ran less at three weeks of age but not at five, seven, nine or eleven weeks.

Calves may respond differently to different planes of nutrition depending on their breed. Holstein Friesian calves showed differences in unrewarded visits to milk and concentrate intake according to plane of nutrition while Jersey calves did not (Byrne *et al.*, 2017). Similarly, Jensen and Holm (2003) found an association between milk allowance and the number and length of unrewarded visits to the feeder in Holstein Friesian and Danish Red calves, but not in Jersey calves. Calves

fed on acidified milk replacer have longer sucking bouts and more pauses in each sucking bout (Todd *et al.*, 2018).

A variety of environmental factors may also influence calf behaviour, including heat stress, with calves in a non-shaded environment changing posture more frequently than those in the shade (Kovacs *et al.*, 2018). One study found that calves drank more milk more slowly when the temperature humidity index was high (Montes *et al.*, 2023). A study conducted in Ohio found that there was no change in daily standing time in young calves between seasons (Hill *et al.*, 2013). A Slovakian study found an effect of season of birth on the behaviour of heifers at twelve months of age (Uhrincat *et al.*, 2022). However, to this authors knowledge, there is no published information on whether the behaviour of pre-weaned calves alters with the natural environment in the UK climate. One study from the UK did show that calves were more likely to seek shelter at higher induced wind speeds (Bell *et al.*, 2019), but there is no work on lying or standing behaviour.

Another factor that will affect a calf's behaviour is the space allowance per calf. Calves housed in groups with a lower space allowance per calf spend less time walking but not less time running than those housed with a higher space allowance (Sutherland *et al.*, 2014). Calves housed in a smaller space do however spend more time running in an arena test (Sutherland *et al.*, 2014). Calves with a reduced space allowance are also more likely to manipulate their bedding substrate (Leruste *et al.*, 2014).

The bedding substrate has been shown to affect the number of lying bouts a calf has, but not total lying time (Sutherland *et al.*, 2017). Calves housed on stones spend less time walking but not running than those housed on sawdust (Sutherland *et al.*, 2014). Calves housed on stones will display more play behaviour on an arena test than those housed on sawdust (Sutherland *et al.*, 2014). Provision of a brush or chopped hay will reduce non-nutritive sucking in pre-weaned calves (Horvath *et al.*, 2020) and a brush will increase eating time in weaned calves (Velasquez-Munoz *et al.*, 2019). In group housed calves, brushes are also associated with higher activity and reduced competition around the feeder (Occhiuto *et al.*, 2025). The provision

of a more complex individual hutch will increase locomotor play in pre-weaned calves (Pempek *et al.*, 2017).

The way calves are handled will also affect a calf's behaviour, calves handled patiently and calmly have been shown to be more friendly and content when undergoing qualitative behaviour assessment (Ellingsen *et al.*, 2014). In contrast, calves handled by nervous or aggressive stockmen have a far more negative mood (Ellingsen *et al.*, 2014). These findings are supported by those of Calderón-Amor *et al.* (2019) who found that calves on farms where the calf manager had a more negative attitude had greater odds of a fearful response during an escape test. Calves handled for forty minutes a day in the first two weeks have a reduced fear of humans and gain more weight pre-weaning (Lürzel *et al.*, 2015).

1.6.3 Using technology to detect behaviour

Changes in behaviour could be used as an early indicator of disease; however, monitoring behaviour manually is labour intensive (Mattachini *et al.*, 2011). In the last thirty years, cattle are increasingly managed in fewer, larger herds and the labour available per animal has decreased (Barkema *et al.*, 2015). Skilled labour is particularly scarce and staff turnover is high. It is therefore becoming increasingly difficult to monitor all animals at once (Berchmans *et al.*, 2014). Thus, the use of sensors to monitor cattle behaviour is increasing (Paudyal *et al.*, 2016). These technologies were initially introduced for oestrus detection in adult cows but are increasingly being explored for disease detection (Eckelkamp and Bewley, 2020). Using automated tools to detect behaviour changes related to disease will aid in the detection of subtle changes, allowing earlier detection of disease (Alsaad *et al.*, 2017). Sensors also have the advantage that they remove any behaviour changes caused by the presence of human observers (Robert *et al.*, 2009), especially as ill animals may attempt to hide their vulnerability (Weary *et al.*, 2009). Studies monitoring activity in cattle have used a variety of sensors to monitor locomotion behaviours, including pedometers (Steensels *et al.*, 2012), ultra-wideband location sensors (Vázquez-Diosdado *et al.*, 2024), global positioning systems (Williams *et al.*, 2016), infrared sensors (Sonntag *et al.*, 2024), leg mounted accelerometers (Thorup *et al.*, 2015), and neck mounted accelerometers (Grinter *et al.*, 2015).

al., 2019). Sensors have been developed for both research and commercial use, and both types are seen in the literature (Borchers *et al.*, 2016). Tri-axial accelerometers measure the angle of tilt and dynamic acceleration and use algorithms to process this information into behavioural data e.g. lying and standing times (Costa *et al.*, 2021). Factors such as sampling interval may affect the accuracy of behavioural classification using accelerometers and there is a trade-off between sampling interval and sensor battery life (Robert *et al.*, 2009). A greater correlation between observed behaviours and results from a leg mounted accelerometers in calves was observed when the accelerometer was attached to the right hind leg rather than either of the left legs (Bonk *et al.*, 2013). Researchers in the UK have also developed a collar mounted accelerometer and gyroscope for behavioural classification in calves (Carslake *et al.*, 2020).

In calves, automated calf feeding systems allow the recording of feeding behaviours (Costa *et al.*, 2021). Automated milk feeders reduce labour requirements by 39% (Sinnott *et al.*, 2021). Estimates of uptake of these systems on dairy farms vary between countries from 2.3% in the UK (Mahendran *et al.*, 2022), 6.6% in Australia (Abuelo *et al.*, 2019) to 16% in Canada (Medrano-Galarza *et al.*, 2017). Thus, there are a variety of sensors available to detect changes in calf behaviour with varying levels of uptake on commercial farms. Ensuring sensors are practical for on-farm use in calves will be essential in the future.

1.6.4 Statistical techniques for using behaviour to detect disease.

A multitude of statistical techniques have been used to process the data from sensors to detect pre-clinical or clinical disease in apparently healthy animals, with varying degrees of success. The best indicators are those that give few false negatives or false positives, i.e. have high sensitivity and specificity (Weary *et al.*, 2009).

There has been increasing interest in using statistical control charts as part of precision livestock farming techniques. A statistical control chart is the graphical representation of a measurement over time and shows the pre-defined limits for that variable (Mertens *et al.*, 2010). A cumulative sum (CUSUM) statistical control chart methodology has been used by several authors in feedlot cattle. Quimby *et*

al. (2001) used CUSUM to detect morbidity using radio frequency technology to detect the time each individual spent in proximity to the feed bunk. These charts were able to detect morbidity 4.1 days earlier than feedlot staff, with a sensitivity of 90%. In contrast Kayser *et al.* (2019) used an electronic feed bunk to record feeding behaviours, and they looked at the frequency and duration of visits as well as dry matter intake. The best performance for a univariate CUSUM chart was dry matter intake with a sensitivity of 66.7% and specificity of 93.5%. However, performance could be improved when using a principal component analysis-based model in a multivariate CUSUM charts using a “either or” rule that included dry matter intake, bunk visit duration, head down duration and the standard deviation of the non-feeding interval. This chart had a sensitivity of 83.3% and a specificity of 94.6%.

CUSUM statistical control charts have also been used in younger animals. Knauer *et al.* (2018) used a CUSUM chart of daily average drinking speed had a sensitivity of 52% and a specificity of 46% when a deviation of 1.5 ml/min from a calves’ normal average speed was considered abnormal. In the same study, combining drinking speed, milk consumption and unrewarded visits improved sensitivity to 74.8%, but specificity was poor at 27.1%. These test characteristics are better than those of the Shewhart method used by Wottlin *et al.* (2021) whose multivariate model for activity traits gave a sensitivity of 47.4% and a specificity of 29.2%. However, there are several important considerations to make when considering the use of statistical control charts, including that raw livestock data often violates the assumptions made by control charts due to its dynamic nature. For example, livestock data tends not to maintain the same statistical properties (mean and covariance of time spent doing a behaviour) over time and frequently contains missing values (Mertens *et al.*, 2010).

Conboy *et al.* (2021) used a best linear unbiased prediction and parallel testing to predict disease using milk feeding behaviours. The sensitivity and specificity for BRD detection were 82% and 26%, respectively. For NCD, the sensitivity and specificity were 79% and 23%, respectively. Interestingly, a multivariable logistic

regression model in older bulls using feeding and lying behaviour to predict disease had a sensitivity of 79% and 81% respectively (Belaid *et al.*, 2019).

Other authors have used machine learning techniques for disease detection. Bowen *et al.* (2021) combined a random forest model for activity and feeding and a model using deviation from a rolling average for lying time to gain an improved sensitivity of 64%, with a 95% specificity for BRD. Chisholm *et al.* (2021) had promising results using a multi-state modelling technique for the detection of BRD in pre-weaned calves with an area under the curve of 0.86. Another machine learning approach, the K-nearest neighbour algorithm had an accuracy of over 90% for identifying calves six days prior to diagnosis of clinical BRD using both the WCHS and TUS (Cantor *et al.*, 2022a). One study used Lasso regularized multivariate logistic regression (Ramezani Gardaloud *et al.*, 2022). When days two and three prior to disease were taken together sensitivity was 71.4% and specificity 95.2% (Ramezani Gardaloud *et al.*, 2022).

A key point to consider is analysing the cost/benefit of improving test characteristics. When assessing how test characteristics change net returns, Theurer *et al.* (2015) found that there was greater benefit of increasing specificity than sensitivity. This is due to the costs associated with unnecessary treatment when specificity is low, as a lower specificity means that there is a greater number of false positives. They found that true prevalence of disease and mortality risk were the main drivers in changes in net returns. This however will only apply if no confirmatory test is used.

A decision support tool will be needed for a disease detection tool to be used on farm (Sun *et al.*, 2021), this is a tool that rather than just giving an alert aids the farmer to make a management decision e.g. what disease to treat for. There are no decision support tools in the literature currently. Furthermore, a farmer friendly user interface is needed in future development (Sun *et al.*, 2021).

1.7 Other technologies to detect disease.

In addition to behaviour monitoring technologies, other technologies for the detection of pyrexia, the autonomic stress response or coughing have been

proposed to aid with disease detection. Various technologies have been proposed to monitor for pyrexia. One such technology is ear tags that incorporate a temperature sensor placed in the external ear canal, however concerns have been raised concerning their accuracy (McCorkell *et al.*, 2014). One such system had a low specificity for BRD diagnosis when TUS was used as the gold standard (Mahendran, 2020).

Pyrexia can also be detected by infrared-thermography cameras which can be used to measure the surface temperature of various areas on a calf (Hoffmann *et al.*, 2016). Schaefer *et al.* (2012) showed that this data can be collected non-invasively by setting up a camera at the water bowl. This paper estimated a sensitivity of 100% and specificity of 87.2% for BRD using their proposed cut off value for eye temperature. In contrast, Bell *et al.* (2020) found that correlation between rectal temperature and infrared thermography measures of the medial canthus of the eye was weak. For technologies such as these which record surface temperatures of calves, the findings of Hill *et al.* (2016) need to be considered, as the temperature of the skin adjacent to the tail vein was found to alter with the ambient temperature. This is likely to also be the case when measuring the temperature of other areas of exposed skin. The rectal temperature will be far closer to the core temperature due to the high vascularity and proximity to major arteries such as the aorta and is thus more biologically relevant. The medial canthus however is far more likely to be affected by external factors and peripheral vasoconstriction in low ambient temperature. In Schaefer *et al.* (2007) sensitivity and specificity of various diagnostic techniques are compared. Using the absolute values from an infrared thermography read out of orbital temperature performed worse than clinical scores and rectal temperature for both sensitivity and specificity. In future, video infrared thermography rather than single image analysis may be of benefit to detect pyrexia due to the larger volume of data provided (Hoffmann *et al.*, 2016). Video infrared thermography has been used to measure calf respiratory rate. Lowe *et al.* (2019a) found a high level of agreement between respiratory rates measured from a video camera recording and that measured by an infrared recording. This was taken further by Yousfi *et al.* (2024) who used computer vision to measure the

respiratory rates of calves from infrared videos with a correlation of 0.98. Computer vision has also been used to measure calf respiratory rates from a colour video camera (Zeng *et al.*, 2023). As yet, there is no research on group-housed calves, however there is some research on “unrestrained” adult cattle in free stalls (Shu *et al.*, 2024), suggesting that measurement of calves may be possible, particularly in the feeder when a calf’s orientation is known.

Microphones have been used to detect coughing in calves. Cough sounds are significantly shorter, with higher amplitude and lower frequency than sounds made by the metal rack in the same pen (Ferrari *et al.*, 2010). However, when used to detect BRD, Vandermeulen *et al.* (2016) found that their algorithm only had a sensitivity of 50.3% although specificity was good at 99.2%. Carpentier *et al.* (2018) used an algorithm with poorer sensitivity of 41.4% and a marginally improved specificity of 99.9%. A wearable sensor to detect coughs using audio information has also been developed but no information is available on its sensitivity or specificity (Vuppalapati *et al.*, 2018).

1.8 Considerations when using technology to detect disease.

The use of technology on dairy farms has increased (Lokhorst *et al.*, 2019). Precision livestock farming (PLF) is the term used for automated monitoring of individual animals (Berckmans, 2006). However, there are many ethical and practical concerns regarding the increased use of PLF for disease detection.

The first of these concerns is around the human animal relationship which develops as the farm staff work with the animals and is dependent on the behaviour of both parties. This relationship is key to the job satisfaction of many farm staff (Kling-Eveillard *et al.*, 2020) and is associated with animal welfare and productivity (Neethirajan, 2023). For over half a century concerns have been raised that the introduction of technology on farm will reduce the relationship between the stockman and their livestock (Buller *et al.*, 2020). With farmers spending an increasing amount of time at the computer their connection with the animal is reduced (Berckmans, 2014) and there is a risk that animals are reduced to a number on a screen (Schillings *et al.*, 2021). Furthermore, if animals become less

habituated to humans (Schillings *et al.*, 2021) it will make handling more stressful for both animals and humans. Kling-Eveillard *et al.* (2020) found that farmers had varying views of the effect of PLF on the human animal relationship. However, overall farmers have a positive view of PLF and many see it as part of improving animal welfare (Kling-Eveillard *et al.*, 2020). It is likely that the farmers role does evolve when technology is introduced as does how they see themselves (Bear and Holloway, 2015). Across the entire supply chain from the farm staff to the consumer the increased use of technology on farm may lead to animals being alienated as it is moving away from the traditional dynamic of “care” on farms (Bossert and Coeckelbergh, 2024). This disruption of the human animal relationship feeds into the instrumentalization of animals which has been raised as a concern when looking at the consequences of these systems (Williamson and Hartley, 2024) and could affect animal welfare (Tuytens *et al.*, 2022).

Farmers may also become de-skilled in animal handling and decision-making (Eastwood *et al.*, 2019b). Previously, concerns over farmers deskilling with the introduction of automatic milking systems have been raised (Driessen and Heutinck, 2015). This could be of particular concern in the event of a system breakdown. Interestingly, these concerns were not shared by the respondents in Eastwood *et al.* (2015) 67% of whom felt that the skill level required on farm had increased and less than 10% felt the skill level required to decreased. Another concern is that farm staff and their advisors may lack the skills and knowledge to enable PLF to be fully utilised on farm (Van Hertem *et al.*, 2017). This is of particular concern in areas with a smaller farming population where less investment in training may be made (Eastwood *et al.*, 2019b). A New Zealand study found that 85% of respondents agreed they would benefit from more support to get the most out of the technology installed on the farm (Eastwood *et al.*, 2015). Furthermore, there is a risk that information is commodified placing less importance on the experience of both farmers and their advisers (Wolf and Wood, 1997). Interactions with advisors are still valued by farmers as they often have farm specific experience (Eastwood *et al.*, 2019a). Particularly if remote access to data is possible then the introduction of technology may lead to a reduction in human interaction on farm

(Rose *et al.*, 2018). Loneliness is a major concern in farming and is associated with a reduction in resilience and an increase in depression in the UK farming community (Kyle *et al.*, 2025). It has also been suggested that PLF tools will lead to more larger farms (Elliot *et al.*, 2023) this could compound this problem.

The uptake of PLF technologies varies between sectors (Groher *et al.*, 2020). There is increasing pressure on farms to be efficient to be profitable (Dawkins, 2025) and thus for farmers to invest in PLF systems a clear business case is needed, this may be particularly difficult in a volatile milk market (Eastwood and Renwick, 2020). For practical application of these technologies on farm, a positive cost benefit and reliable independently validated functionality is required (Richeson, 2020).

Consumers are concerned that the introduction of further technology on farms is part of an increase in “factory farming” (Henchion *et al.*, 2022). For example, if technology is introduced to detect disease that could in turn lead to automated medicating of e.g. feed or water and allow increased intensification (Coghlan and Parker, 2023). If systems reached this level of automation then there would be a high cost to any mistakes the system makes (Coghlan and Parker, 2023).

Furthermore, if humans remain responsible for treatment there is still a risk that disease detection tools can reduce welfare. If these tools only leading to action when disease has been detected animal management may be reduced to purely acting on alerts and improvements to the animal management to foster positive welfare are not made (Schillings *et al.*, 2021).

Tuytens *et al.* (2022) identified further risks to animal welfare posed by these technologies including technical failures, poor external validation, focussing on what can be measured, not what is meaningful and over or under reliance on PLF. This seminal work on the welfare risks of PLF covers these areas to a far greater degree than there is scope for here. Currently the research mainly frames technologies positively when it comes to animal welfare and care should be taken by authors not to introduce bias to the literature (Weary and von Keyserlingk, 2023).

1.9 Biomarkers

The use of biomarkers to detect disease has been an area of considerable research. In this section, biomarkers that have been researched for the detection of BRD and NCD in calves will be discussed.

An increase in the acute phase protein haptoglobin in serum in the first week of life has been associated with an increased chance of mortality in the first 4 months of life, however sensitivity was poor (Murray *et al.*, 2014). Elevated levels of serum haptoglobin at arrival to a veal facility for rearing (less than seven days old) has been associated with a greater hazard of morbidity and lower ADG (Goetz *et al.*, 2021). Bovine respiratory disease is associated with increased serum haptoglobin and lipopolysaccharide binding protein (Idoate *et al.*, 2015). Serum haptoglobin concentration is correlated with WCHS (Joshi *et al.*, 2018). Moisa *et al.* (2019) found that plasma haptoglobin had a sensitivity of 46.4% and specificity of 81.6% when using TUS and auscultation in combination as the gold standard. El-Deeb *et al.* (2020) reported improved test characteristics with a sensitivity of 81.2% and specificity of 100% for serum haptoglobin. The use of serum haptoglobin alongside other measures to build a model for disease has been suggested (Burciaga-Robles *et al.*, 2009). Plasma haptoglobin has been combined with TUS as a gold standard when developing a new scoring system for veal calves (Berman *et al.*, 2022). A challenge in the use of serum haptoglobin as a biomarker is that there may be breed differences. Johnston *et al.* (2016a) showed that haptoglobin concentrations during gradual weaning were significantly different between Holstein-Friesian and Jersey bull calves.

Another acute phase protein, serum amyloid A (SAA) has been shown to be increased in serum in BRD cases (Fathi *et al.*, 2011, Orro *et al.*, 2011) and is correlated with WCHS (Joshi *et al.*, 2018). El-Deeb *et al.* (2020) found that serum SAA has a sensitivity of 89.9% and a specificity of 80% in feed-lot cattle, however the number of control calves was low in that study. Lowie *et al.* (2025) found that SAA was not associated with lung consolidation or the Californian respiratory score. The systemic review by Abdallah *et al.* (2016) noted the need for greater standardization in reporting and BRD definitions to enable conclusions to be drawn

on the use of haptoglobin and SAA in the diagnosis of BRD. There also may be a significant delay between disease and an increase in SAA (Martin *et al.*, 2022). Interleukin-6 (IL-6) is an early inflammatory biomarker that has been of interest in calf disease work. Serum IL-6 has been shown to have a sensitivity of 77% and specificity of 88% for the detection of calves with a prolonged recovery period following NCD (Fischer *et al.*, 2016). Circulating IL-6 has also been identified as being higher in calves with pneumonia (Kabu *et al.*, 2016). There are research groups developing biosensors for human IL-6 (Huang *et al.*, 2013). Both bovine respiratory syncytial virus and *M. haemolytica* artificial infections have been shown to stimulate an increase in the expression of IL-17, IL-21, and IL-22 in the lung tissue of young cattle (McGill *et al.*, 2016). *M. haemolytica* also increased the expression of IL-1 β , tumour necrosis factor- α (TNF- α) and IL-8 in the airways. In contrast in *M. bovis* infection TNF- α , IL-4, IL-10 and interferon- γ are upregulated in the lungs (Rodriguez *et al.*, 2015). However, Valsala *et al.* (2017) found that in peripheral blood mononuclear cells IL-6 was upregulated and IL-1 and TNF- α was not. Serum IL-8 has also been associated with intestinal epithelial damage in calves with NCD (Ok *et al.*, 2020). Calves with NCD have higher procalcitonin, haptoglobin and TNF- α (Sayar and Keles, 2024). However, procalcitonin has poor diagnostic accuracy for BRD with a sensitivity of 50% and specificity of 72% (Sala *et al.*, 2024). While inflammatory markers are an interesting area of research there is still much work to be done to confirm whether local expression of inflammatory markers translates to increased levels of the same markers in the circulation, and whether this can improve the detection of early disease or disease prognosis.

A variety of other circulating molecules have been suggested as potential biomarkers. Lactate is higher in calves that go on to develop BRD than those that do not (Zeineldin *et al.*, 2017). Camkerten *et al.* (2010) found that not only is lactate higher in calves with BRD compared to healthy calves, but that it was also a prognostic indicator. Plasma diamine oxidase activity has been suggested as a measure of mucosal damage secondary to NCD (Fukuda *et al.*, 2019). However, when assessed as a diagnostic marker for the intestinal pathogen *C. parvum*, specificity is poor at 34.8% (Tsukano *et al.*, 2020). Plasma histidine has better test

characteristics with a sensitivity of 88.9% and specificity of 82.6%. All the above biomarkers require a blood sample to be taken to measure them, thus they are not at present candidates to be measured regularly to detect disease.

Volatile organic compounds have also been suggested as a possible biomarker. Four of these compounds have been shown to be significantly different in nasal swabs between healthy and BRD affected cattle (Maurer *et al.*, 2018). However, a mass spectrometer is currently required for this work, therefore there is still significant work to be done before application on farm will be possible.

1.9.1 Biomarkers in Saliva

Saliva can be sampled non-invasively making it an interesting candidate for measuring biomarkers (Contreras-Aguilar *et al.*, 2019). While most saliva components are produced by the salivary gland, a portion come from the circulation, thus biomarkers may appear in the saliva (Lamy and Mau, 2012). Contreras-Aguilar *et al.* (2019) found that alpha-amylase, cortisol, lactate, and uric acid were all significantly higher in the saliva of cows with mastitis than in those without mastitis. A recent study used spectroscopy on saliva to detect Johnne's disease positive cows (Behdad *et al.*, 2024). C-reactive protein has been identified in porcine saliva and has been shown to be associated with inflammatory conditions (Gutierrez *et al.*, 2009). These measures would all require advanced techniques e.g. spectroscopy and enzyme linked immunosorbent assay; thus, they are not candidates for rapid on farm diagnostics or being automatically measured. Dehydration is a common finding in calves with NCD (Trefz *et al.*, 2013) and is a significant cause of their death (Hulbert and Moisa, 2016). In human subjects, both unstimulated and stimulated saliva flow from the parotid gland has been shown to be decreased by dehydration (Ship and Fischer, 1997). However, no correlation was found between flow rate and metabolic measures of dehydration (Ship and Fischer, 1997). Saliva osmolarity has been shown to be highly correlated with body mass loss after exercise and thus dehydration (Walsh *et al.*, 2004, Smith *et al.*, 2012). In children with cerebral palsy saliva osmolarity was correlated with plasma osmolarity (Santos *et al.*, 2011). A handheld device to measure saliva osmolarity has been used to detect dehydration in healthy children (Faidah *et al.*, 2021).

However, the study by Taylor *et al.* (2012) found that sensitivity was poor for individuals over 6% dehydrated at 42%, while sensitivity for those between 3 and 6% dehydrated was 64%. However, when a simpler cut-off was used the area under the curve was 0.87 for 3% dehydration and 0.83 for 6% dehydration. Munoz *et al.* (2013) found that in adult men exposed to heat, saliva osmolarity has a sensitivity of 86% and specificity of 91% for the detection of $\geq 2\%$ dehydration. Saliva conductivity has also been used and is consistent with serum osmolarity (Lu *et al.*, 2019). To date there is no research exploring saliva osmolarity or conductivity in farm animal species. While the evidence is mixed for the association of saliva osmolarity and dehydration overall saliva osmolarity appears to be correlated with serum osmolarity. Saliva conductivity is correlated with saliva osmolarity and therefore shows some promise in the detection of dehydration. Furthermore, saliva conductivity can be measured using small volumes in a portable metre and therefore there may be potential for the development of an on-farm tool.

In calves with BRD, respiratory acidosis has been identified and is associated with the severity of disease (Šoltésová *et al.*, 2015). Calves with NCD frequently develop lactic acidosis and a plasma anion gap is seen (Bednarski and Kupczyński, 2015). Fluid therapy needs to be instigated early to reduce the likelihood of mortality (Smith and Berchtold, 2014). Salivary pH has been shown to be associated with blood cholesterol in humans (Suzuki *et al.*, 2020). Again, in humans, the salivary anion gap has been found to be correlated with plasma anion gap and had a sensitivity and specificity for acidosis of 87.7% and 84.2 % respectively (Hamada *et al.*, 2013). Thus, ions pass into saliva suggesting that saliva may also reflect any alterations in the serum pH and calves affected by BRD and NCD may both have difference in serum pH due to the presence of acidosis. A study of critically ill calves found that low venous blood pH was the only lab parameter associated with mortality in critically ill calves (Trefz *et al.*, 2017). Like conductivity pH can be measured with a portable metre. Thus, there is a variety of biomarkers that may be measured in saliva. However, due to the ease of measurement saliva conductivity and pH have particular potential, especially for NCD where dehydration is a common sequel to disease.

1.10 Conclusion

In conclusion, calf diseases are a significant concern for the agricultural industry due to high mortality, long-term effects, and cost. Early detection of disease also allows for a reduction in antimicrobial use, as treatments can be implemented earlier, hence reducing the need for second treatments and the spread of disease. Overall, early detection will allow for lower morbidity and mortality, reduced long term effects and reduced cost to the farm.

Disease affects lying, feeding, and walking behaviours in calves and this may be used in models to detect disease. However, several other factors such as age and sex also affect behaviour. A variety of statistical methods have been used with mixed results. For disease detection technologies to be implemented on farm, both accuracy and compatibility between technologies will need to improve.

Biomarkers for disease is an area of considerable research, but far less work has explored the use of salivary biomarkers that allow non-invasive sampling. In calves with NCD, both dehydration and metabolic acidosis are a concern. The use of saliva osmolarity, and pH has been an area of research in humans but has not yet been studied in calves.

1.11 Thesis plan

Chapter 1 has identified some gaps in the literature which need to be addressed. Firstly, we do not have a full understanding of how intrinsic calf factors will affect the calf's milk feeding and activity parameters as measured by available technologies. Secondly, the effect of disease on calf behaviour in dairy-beef and bull calves is not well understood. Finally, the potential for rapid diagnostics on the readily sampled bodily fluid saliva has not been explored.

The aim of this thesis was to identify measures that has potential to be utilised as part of an automated disease detection system in group housed artificially reared pre-weaned limit fed dairy-bred calves.

The objectives of the thesis are as follows:

- Understand the association between intrinsic calf factors and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves to inform pairing criteria and factors for inclusion in mixed models when analysing the association between these variables and disease.
- Understand the association between bovine respiratory disease and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.
- Understand the association between neonatal calf diarrhoea with or without dehydration and saliva conductivity and pH in group housed artificially reared pre-weaned limit fed dairy-bred calves.
- Understand the association between saliva conductivity and pH and physiological indicators of dehydration or acidosis in blood from group housed artificially reared pre-weaned limit fed dairy-bred calves.
- Understand the association between neonatal calf diarrhoea and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.

1.12 Bibliography

- ABDALLAH, A., HEWSON, J., FRANCOZ, D., SELIM, H. & BUCZINSKI, S. 2016. Systematic Review of the Diagnostic Accuracy of Haptoglobin, Serum Amyloid A, and Fibrinogen versus Clinical Reference Standards for the Diagnosis of Bovine Respiratory Disease. *Journal of Veterinary Internal Medicine*, 30, 1356-1368.
- ABDELFATTAH, E. M., KAROUSA, M. M., LAY, D. C., JR., MARCHANT-FORDE, J. N. & EICHER, S. D. 2018. Short communication: Effect of age at group housing on behavior, cortisol, health, and leukocyte differential counts of neonatal bull dairy calves. *Journal of Dairy Science*, 101, 596-602.
- ABDELFATTAH, E. M., SCHUTZ, M. M., LAY, D. C., JR., MARCHANT-FORDE, J. N. & EICHER, S. D. 2013. Effect of group size on behavior, health, production, and welfare of veal calves. *Journal of Animal Science*, 91, 5455-5465.
- ABUELO, A., CULLENS, F. & BRESTER, J. L. 2021. Effect of preweaning disease on the reproductive performance and first-lactation milk production of heifers in a large dairy herd. *Journal of Dairy Science*, 104, 7008-7017.

- ABUELO, A., HAVRLANT, P., WOOD, N. & HERNANDEZ-JOVER, M. 2019. An investigation of dairy calf management practices, colostrum quality, failure of transfer of passive immunity, and occurrence of enteropathogens among Australian dairy farms. *Journal of Dairy Science*, 102, 8352-8366.
- ADAMS, E. A. & BUCZINSKI, S. 2016. Short communication: Ultrasonographic assessment of lung consolidation postweaning and survival to the first lactation in dairy heifers. *Journal of Dairy Science*, 99, 1465-1470.
- AGHAKESHMIRI, F., AZIZADEH, M., FARZANEH, N. & GORJIDOOZ, M. 2017. Effects of neonatal diarrhea and other conditions on subsequent productive and reproductive performance of heifer calves. *Veterinary Research Communications*, 41, 107-112.
- AGRICULTURE AND HORTICULTURE DEVELOPMENT BOARD 2020. GB Dairy Calf Strategy 2020–2023.
https://projectblue.blob.core.windows.net/media/Default/Dairy/Publications/DairyCalfStrategy_200826_WEB.pdf
- ALGERS, B. Injury and disease. Proceedings of Global conference on Animal Welfare: an OIE initiative. Proceedings of Global conference on Animal Welfare: an OIE initiative, 2004. OIE Paris, 23-25.
- ALSAAOD, M., KREDEL, R., HOFER, B. & STEINER, A. 2017. Technical note: Validation of a semi-automated software tool to determine gait-cycle variables in dairy cows. *Journal of Dairy Science*, 100, 4897-4902.
- ALY, S. S., LOVE, W. J., WILLIAMS, D. R., LEHENBAUER, T. W., VAN EENENNAAM, A., DRAKE, C., KASS, P. H. & FARVER, T. B. 2014. Agreement between bovine respiratory disease scoring systems for pre-weaned dairy calves. *Animal Health Research Reviews*, 15, 148-50.
- ANIMAL AND PLANT HEALTH AGENCY & SCOTTISH AGRICULTURE COLLEGE VETERINARY SERVICES 2017. Alimentary and respiratory disease trends for calves, 2011 to 2016. *Veterinary Record*, 180, 144-145.
- BACH, A. 2011. Associations between several aspects of heifer development and dairy cow survivability to second lactation. *Journal of Dairy Science*, 94, 1052-7.
- BABKINE, M. & BLOND, L. 2009. Ultrasonography of the Bovine Respiratory System and Its Practical Application. *Veterinary Clinics of North America: Food Animal Practice*, 25, 633-649.
- BALLOU, M. A., COBB, C. J., HULBERT, L. E. & CARROLL, J. A. 2011. Effects of intravenous *Escherichia coli* dose on the pathophysiological response of colostrum-fed Jersey calves. *Veterinary Immunology and Immunopathology*, 141, 76-83.

- BARKEMA, H. W., VON KEYSERLINGK, M. A. G., KASTELIC, J. P., LAM, T. J. G. M., LUBY, C., ROY, J. P., LEBLANC, S. J., KEEFE, G. P. & KELTON, D. F. 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. *Journal of Dairy Science*, 98, 7426-7445.
- BARKLEY, J. A., PEMPEK, J. A., BOWMAN, A. S., NOLTING, J. M., LEE, J., LEE, S. & HABING, G. G. 2021. Longitudinal health outcomes for enteric pathogens in preweaned calves on Ohio dairy farms. *Preventive Veterinary Medicine*, 190, 105323.
- BARUCH, J., CERNICCHIARO, N., CULL, C. A., LECHTENBERG, K. F., NICKELL, J. S. & RENTER, D. G. 2019. Performance of multiple diagnostic methods in assessing the progression of bovine respiratory disease in calves challenged with infectious bovine rhinotracheitis virus and Mannheimia haemolytica. *Journal of Animal Science*, 97, 2357-2367.
- BAXTER-SMITH, K., MORE, J. & HYDE, R. 2022. Use of thoracic ultrasound on Scottish dairy cattle farms to support the diagnosis and treatment of bovine respiratory disease in calves. *Veterinary Record*, 190, e939.
- BAUMAN, C. A., BARKEMA, H. W., DUBUC, J., KEEFE, G. P. & KELTON, D. F. 2016. Identifying management and disease priorities of Canadian dairy industry stakeholders. *Journal of Dairy Science*, 99, 10194-10203.
- BEAR, C. & HOLLOWAY, L. 2015. Country Life: Agricultural Technologies and the Emergence of New Rural Subjectivities. *Geography Compass*, 9, 303-315.
- BEDNARSKI, M. & KUPCZYŃSKI, R. 2015. Analysis of acid–base disorders in calves with lactic acidosis using a classic model and strong ion approach. *Turkish Journal of Veterinary and Animal Sciences*, 39, 615-620.
- BEHDAD, S., PAKDEL, A. & MASSUDI, R. 2024. Saliva NIR spectroscopy and Aquaphotomics: a novel diagnostic approach to Paratuberculosis in dairy cattle. *Frontiers in Cellular and Infection Microbiology*, 14.
- BEHERA, S. 2025. *Dairy calf registrations: Lowest on record* [Online]. <https://ahdb.org.uk/news/dairy-calf-registrations-lowest-on-record>: Agriculture and Horticulture Development Board. [Accessed 21/03/2025 2025].
- BELAID, M. A., RODRIGUEZ-PRADO, M., CHEVAUX, E. & CALSAMIGLIA, S. 2019. The Use of an Activity Monitoring System for the Early Detection of Health Disorders in Young Bulls. *Animals*, 9, 924.
- BELAID, M. A., RODRIGUEZ-PRADO, M., RODRIGUEZ-PRADO, D. V., CHEVAUX, E. & CALSAMIGLIA, S. 2020. Using behavior as an early predictor of sickness in veal calves. *Journal of Dairy Science*, 103, 1874-1883.

- BELL, D. J., DUTHIE, C. A., MASON, C. S., HANCOCK, A., PENNY, C., ODEYEMI, I., BARTRAM, D. J. & HASKELL, M. J. 2024. Developing a tool to assess the health-related quality of life in calves with respiratory disease: tool refinement and construct validity testing. *animal*, 18, 101215.
- BELL, D. J., HASKELL, M. J., MACRAE, A. I., MITCHELL, M. A., JENNINGS, A. & MASON, C. S. 2019. Behavioural responses of dairy calves to temperature and wind. 53rd Annual Congress of the ISAE, 2019. Wageningen Academic Publishers.
- BELL, D. J., MACRAE, A. I., MITCHELL, M. A., MASON, C. S., JENNINGS, A. & HASKELL, M. J. 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*, 131, 259-265.
- BELL, D. J., VIGORS, B., DUTHIE, C. A., BARTRAM, D. J., HANCOCK, A., ODEYEMI, I., PENNY, C. & HASKELL, M. J. 2023. Developing a tool to assess the health-related quality of life in calves with respiratory disease: content validation. *animal*, 17, 100702.
- BERCKMANS, D. 2006. Automatic on-line monitoring of animals by Precision Livestock Farming. Leiden, The Netherlands: Wageningen Academic.
- BERCKMANS, D. 2014. Precision livestock farming technologies for welfare management in intensive livestock systems. *OIE Revue Scientifique et Technique*, 33, 189-196.
- BERMAN, J., FRANCOZ, D., ABDALLAH, A., DUFOUR, S. & BUCZINSKI, S. 2021. Evaluation of inter-rater agreement of the clinical signs used to diagnose bovine respiratory disease in individually housed veal calves. *Journal of Dairy Science*, 104, 12053-12065.
- BERMAN, J., FRANCOZ, D., ABDALLAH, A., DUFOUR, S. & BUCZINSKI, S. 2022. Development and validation of a clinical respiratory disease scoring system for guiding treatment decisions in veal calves using a Bayesian framework. *Journal of Dairy Science*, 105, 9917-9933.
- BERMAN, J., FRANCOZ, D., DUFOUR, S. & BUCZINSKI, S. 2019. Bayesian estimation of sensitivity and specificity of systematic thoracic ultrasound exam for diagnosis of bovine respiratory disease in pre-weaned calves. *Preventive Veterinary Medicine*, 162, 38-45.
- BERMAN, J., MASSEAU, I., FECTEAU, G., BUCZINSKI, S. & FRANCOZ, D. 2020. Comparison between thoracic ultrasonography and thoracic radiography for the detection of thoracic lesions in dairy calves using a two-stage Bayesian method. *Preventive Veterinary Medicine*, 184, 105153.

- BERNIER GOSELIN, V., PERRETEEN, V., COLLAUD, A., SCHÜPBACH-REGULA, G. & MEYLAN, M. 2025. Antimicrobial resistance in *Escherichia coli* isolates from the calf environment on Swiss dairy farms. *Research in Veterinary Science*, 196, 105893.
- BLACK, R. A., WHITLOCK, B. K. & KRAWCZEL, P. D. 2017. Effect of maternal exercise on calf dry matter intake, weight gain, behavior, and cortisol concentrations at disbudding and weaning. *Journal of Dairy Science*, 100, 7390-7400.
- BOCCARDO, A., FERRARO, S., SALA, G., FERRULLI, V., PRAVETTONI, D. & BUCZINSKI, S. 2023. Bayesian evaluation of the accuracy of a thoracic auscultation scoring system in dairy calves with bronchopneumonia using a standard lung sound nomenclature. *Journal of Veterinary Internal Medicine*, 37, 1603-1613.
- BOCCARDO, A., SALA, G., COPPOLETTA, E., BRONZO, V., PROSERPIO, M., BELLOLI, A. G. & PRAVETTONI, D. 2019. Frequency and severity of neonatal calf diarrhea cases treated with a standard veterinary hospital protocol do not affect heifer reproduction performance and first lactation production. *Livestock Science*, 230, 103836.
- BONIZZI, S., GISLON, G., BRASCA, M., MORANDI, S., SANDRUCCI, A. & ZUCALI, M. 2022. Air Quality, Management Practices and Calf Health in Italian Dairy Cattle Farms. *Animals* [Online], 12.
- BONK, S., BURFEIND, O., SUTHAR, V. S. & HEUWIESER, W. 2013. Technical note: evaluation of data loggers for measuring lying behavior in dairy calves. *Journal of Dairy Science*, 96, 3265-71.
- BORCHERS, M. R., CHANG, Y. M., TSAI, I. C., WADSWORTH, B. A. & BEWLEY, J. M. 2016. A validation of technologies monitoring dairy cow feeding, ruminating, and lying behaviors. *Journal of Dairy Science*, 99, 7458-7466.
- BORDERAS, T. F., DE PASSILLÉ, A. M. & RUSHEN, J. 2008. Behavior of dairy calves after a low dose of bacterial endotoxin. *Journal of Animal Science*, 86, 2920-2927.
- BORDERAS, T. F., RUSHEN, J., VON KEYSERLINGK, M. A. & DE PASSILLE, A. M. 2009. Automated measurement of changes in feeding behavior of milk-fed calves associated with illness. *Journal of Dairy Science*, 92, 4549-54.
- BOSSERT, L. N. & COECKELBERGH, M. 2024. From MilkingBots to RoboDolphins: How AI changes human-animal relations and enables alienation towards animals. *Humanities and Social Sciences Communications*, 11, 920.
- BOULTON, A. C., RUSHTON, J. & WATHES, D. C. 2017. An empirical analysis of the cost of rearing dairy heifers from birth to first calving and the time taken to repay these costs. *Animal*, 11, 1372-1380.

BOWEN, J. M., HASKELL, M. J., MILLER, G. A., MASON, C. S., BELL, D. J. & DUTHIE, C. A. 2021. Early prediction of respiratory disease in preweaning dairy calves using feeding and activity behaviors. *Journal of Dairy Science*, 104, 12009-12018.

BRICKELL, J. S., MCGOWAN, M. M., PFEIFFER, D. U. & WATHES, D. C. 2009. Mortality in Holstein-Friesian calves and replacement heifers, in relation to body weight and IGF-I concentration, on 19 farms in England. *Animal*, 3, 1175-1182.

BRICKELL, J. S. & WATHES, D. C. 2011. A descriptive study of the survival of Holstein-Friesian heifers through to third calving on English dairy farms. *Journal of Dairy Science*, 94, 1831-8.

BROWN, A. J., SCOLEY, G., O'CONNELL, N., GORDON, A. & MORRISON, S. 2023. The impact of plane of nutrition and ambient temperature on the energy utilisation, performance, health and behaviour of newborn dairy-bred bull calves. *Animal - science proceedings*, 14, 399-400.

BRSCIC, M., DAM OTTEN, N., CONTIERO, B. & KIRCHNER, M. K. 2019. Investigation of a Standardized Qualitative Behaviour Assessment and Exploration of Potential Influencing Factors on the Emotional State of Dairy Calves. *Animals*, 9.

BUČKOVÁ, K., ŠÁROVÁ, R., MORAVCSÍKOVÁ, Á. & ŠPINKA, M. 2021. The effect of pair housing on dairy calf health, performance, and behavior. *Journal of Dairy Science*, 104, 10282-10290.

BUCZINSKI, S., ACHARD, D. & TIMSIT, E. 2021. Effects of calfhod respiratory disease on health and performance of dairy cattle: A systematic review and meta-analysis. *Journal of Dairy Science*, 104, 8214-8227.

BUCZINSKI, S., BORRIS, M. E. & DUBUC, J. 2018a. Herd-level prevalence of the ultrasonographic lung lesions associated with bovine respiratory disease and related environmental risk factors. *Journal of Dairy Science*, 101, 2423-2432.

BUCZINSKI, S., BUATHIER, C., BÉLANGER, A. M., MICHAUX, H., TISON, N. & TIMSIT, E. 2018b. Inter-rater agreement and reliability of thoracic ultrasonographic findings in feedlot calves, with or without naturally occurring bronchopneumonia. *Journal of Veterinary Internal Medicine*, 32, 1787-1792.

BUCZINSKI, S., FAURE, C., JOLIVET, S. & ABDALLAH, A. 2016a. Evaluation of inter-observer agreement when using a clinical respiratory scoring system in pre-weaned dairy calves. *New Zealand Veterinary Journal*, 64, 243-7.

BUCZINSKI, S., FECTEAU, G., DUBUC, J. & FRANCOZ, D. 2018c. Validation of a clinical scoring system for bovine respiratory disease complex diagnosis in preweaned dairy calves using a Bayesian framework. *Preventive Veterinary Medicine*, 156, 102-112.

- BUCZINSKI, S., FORTE, G. & BELANGER, A. M. 2013. Short communication: ultrasonographic assessment of the thorax as a fast technique to assess pulmonary lesions in dairy calves with bovine respiratory disease. *Journal of Dairy Science*, 96, 4523-8.
- BUCZINSKI, S., FORTE, G., FRANCOZ, D. & BELANGER, A. M. 2014. Comparison of thoracic auscultation, clinical score, and ultrasonography as indicators of bovine respiratory disease in preweaned dairy calves. *Journal of Veterinary Internal Medicine*, 28, 234-42.
- BUCZINSKI, S., MENARD, J. & TIMSIT, E. 2016b. Incremental Value (Bayesian Framework) of Thoracic Ultrasonography over Thoracic Auscultation for Diagnosis of Bronchopneumonia in Preweaned Dairy Calves. *Journal of Veterinary Internal Medicine*, 30, 1396-401.
- BUCZINSKI, S. & PARDON, B. 2020. Bovine Respiratory Disease Diagnosis: What Progress Has Been Made in Clinical Diagnosis? *Veterinary Clinics of North America: Food Animal Practice*, 36, 399-423.
- BUCZINSKI, S., T, L. O. & DENDUKURI, N. 2015. Bayesian estimation of the accuracy of the calf respiratory scoring chart and ultrasonography for the diagnosis of bovine respiratory disease in pre-weaned dairy calves. *Preventive Veterinary Medicine*, 119, 227-31.
- BULLER, H., BLOKHUIS, H., LOKHORST, K., SILBERBERG, M. & VEISSIER, I. 2020. Animal Welfare Management in a Digital World. *Animals* [Online], 10.
- BURCIAGA-ROBLES, L. O., HOLLAND, B. P., STEP, D. L., KREHBIEL, C. R., MCMILLEN, G. L., RICHARDS, C. J., SIMS, L. E., JEFFERS, J. D., NAMJOU, K. & MCCANN, P. J. 2009. Evaluation of breath biomarkers and serum haptoglobin concentration for diagnosis of bovine respiratory disease in heifers newly arrived at a feedlot. *American Journal of Veterinary Research*, 70, 1291-8.
- BURDICK, N. C., CARROLL, J. A., HULBERT, L. E., DAILEY, J. W., BALLOU, M. A., RANDEL, R. D., WILLARD, S. T., VANN, R. C. & WELSH, T. H. 2011. Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls. *Innate Immunity*, 17, 355-364.
- BYRNE, C. J., FAIR, S., ENGLISH, A. M., JOHNSTON, D., LONERGAN, P. & KENNY, D. A. 2017. Effect of milk replacer and concentrate intake on growth rate, feeding behaviour and systemic metabolite concentrations of pre-weaned bull calves of two dairy breeds. *Animal*, 11, 1531-1538.
- CALDERON-AMOR, J., BEAVER, A., VON KEYSERLINGK, M. A. G. & GALLO, C. 2020. Calf- and herd-level factors associated with dairy calf reactivity. *Journal of Dairy Science*, 103, 4606-4617.

CALDERÓN-AMOR, J. & GALLO, C. 2020. Dairy Calf Welfare and Factors Associated with Diarrhea and Respiratory Disease among Chilean Dairy Farms. *Animals*, 10, 1115.

CAMKERTEN, I., KAZIM BORK, M., KALINBACAK, A., BORAZAN, G. O., SAHIN, T. & SINAN AKTA, M. 2010. Clinical Utility of Lactate in Calves with Bovine Respiratory Disease. *Journal of Animal and Veterinary Advances*, 9, 1955-1957.

CANTOR, M. C., CASELLA, E., SILVESTRI, S., RENAUD, D. L. & COSTA, J. H. C. 2022a. Using Machine Learning and Behavioral Patterns Observed by Automated Feeders and Accelerometers for the Early Indication of Clinical Bovine Respiratory Disease Status in Preweaned Dairy Calves. *Frontiers in Animal Science*, 3.

CANTOR, M. C. & COSTA, J. H. C. 2022. Daily behavioral measures recorded by precision technology devices may indicate bovine respiratory disease status in preweaned dairy calves. *Journal of Dairy Science*, 105, 6070-6082.

CANTOR, M. C., RENAUD, D. L., NEAVE, H. W. & COSTA, J. H. C. 2022b. Feeding behavior and activity levels are associated with recovery status in dairy calves treated with antimicrobials for Bovine Respiratory Disease. *Scientific Reports*, 12, 4854.

CARPENTIER, L., BERCKMANS, D., YOUSSEF, A., BERCKMANS, D., VAN WATERSCHOOT, T., JOHNSTON, D., FERGUSON, N., EARLEY, B., FONTANA, I., TULLO, E., GUARINO, M., VRANKEN, E. & NORTON, T. 2018. Automatic cough detection for bovine respiratory disease in a calf house. *Biosystems Engineering*, 173, 45-56.

CARROLL, A., BLEACH, E. & WILLIAMS, L. 2022. 91. The influence of birth weight on dairy Holstein heifer calf health and growth prior to weaning. *Animal - science proceedings*, 13, 63-64.

CARROLL, J. A., BURDICK SANCHEZ, N. C., HULBERT, L. E., BALLOU, M. A., DAILEY, J. W., CALDWELL, L. C., VANN, R. C., WELSH, T. H. & RANDEL, R. D. 2015. Sexually dimorphic innate immunological responses of pre-pubertal Brahman cattle following an intravenous lipopolysaccharide challenge. *Veterinary Immunology and Immunopathology*, 166, 108-115.

CARSLAKE, C., VAZQUEZ-DIOSDADO, J. A. & KALER, J. 2020. Machine Learning Algorithms to Classify and Quantify Multiple Behaviours in Dairy Calves Using a Sensor: Moving beyond Classification in Precision Livestock. *Sensors* 21, 1-14.

CHAPMAN, C. E., ERICKSON, P. S., QUIGLEY, J. D., HILL, T. M., BATEMAN, H. G., SUAREZ-MENA, F. X. & SCHLOTTERBECK, R. L. 2016. Effect of milk replacer program on calf performance and digestion of nutrients with age of the dairy calf. *Journal of Dairy Science*, 99, 2740-2747.

- CHISHOLM, E., SCOLEY, G. & LAMROCK, F. 2021. 106. Using a multi-state modelling (MSM) approach to predict dairy calf health status using on-farm precision technology data. *Animal - Science Proceedings*, 12, 85.
- CLINQUART, J., LOWIE, T., JOURQUIN, S., BOKMA, J. & PARDON, B. 2025. Association of Lung Consolidation Depth With Pathogens Isolated From Bronchoalveolar Lavage Fluid in Calves With Clinical Signs of Respiratory Disease. *Journal of Veterinary Internal Medicine*, 39, e70120.
- CLOSS, G. & DECHOW, C. 2017. The effect of calf-hood pneumonia on heifer survival and subsequent performance. *Livestock Science*, 205, 5-9.
- COGHLAN, S. & PARKER, C. 2023. Harm to Nonhuman Animals from AI: a Systematic Account and Framework. *Philosophy & Technology*, 36, 25.
- CONBOY, M. H., WINDER, C. B., MEDRANO-GALARZA, C., LEBLANC, S. J., HALEY, D. B., COSTA, J. H. C., STEELE, M. A. & RENAUD, D. L. 2021. Associations between feeding behaviors collected from an automated milk feeder and disease in group-housed dairy calves in Ontario: A cross-sectional study. *Journal of Dairy Science*, 104, 10183-10193.
- CONLY, J. & JOHNSTON, B. 2005. Where are all the new antibiotics? The new antibiotic paradox. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 16, 159-60.
- CONTRERAS-AGUILAR, M. D., MONKEVICIENE, I., CERON, J. J., SILINSKAS, I., VALLEJO-MATEO, P. J., TECLES, F., MARTINEZ-SUBIELA, S., TVARIJONAVICIUTE, A. & ZELVYTE, R. 2019. Biochemical changes in saliva of cows with inflammation: A pilot study. *Research in Veterinary Science*, 124, 383-386.
- COSTA, J. H. C., CANTOR, M. C. & NEAVE, H. W. 2021. Symposium review: Precision technologies for dairy calves and management applications. *Journal of Dairy Science*, 104, 1203-1219.
- CRAMER, C., PROUDFOOT, K. & OLLIVETT, T. 2020. Automated Feeding Behaviors Associated with Subclinical Respiratory Disease in Prewaned Dairy Calves. *Animals*, 10, 1-9.
- CRAMER, M. C. & OLLIVETT, T. L. 2019. Growth of preweaned, group-housed dairy calves diagnosed with respiratory disease using clinical respiratory scoring and thoracic ultrasound-A cohort study. *Journal of Dairy Science*, 102, 4322-4331.
- CRAMER, M. C., OLLIVETT, T. L. & STANTON, A. L. 2016. Associations of behavior-based measurements and clinical disease in preweaned, group-housed dairy calves. *Journal of Dairy Science*, 99, 7434-7443.
- CRAMER, M. C., PROUDFOOT, K. L. & OLLIVETT, T. L. 2019. Short communication: Behavioral attitude scores associated with bovine respiratory disease identified

using calf lung ultrasound and clinical respiratory scoring. *Journal of Dairy Science*, 102, 6540-6544.

CRAMER, M. C. & STANTON, A. L. 2015. Associations between health status and the probability of approaching a novel object or stationary human in preweaned group-housed dairy calves. *Journal of Dairy Science*, 98, 7298-308.

CUEVAS-GOMEZ, I., MCGEE, M., SANCHEZ, J. M., O'RIORDAN, E., BYRNE, N., MCDANELD, T. & EARLEY, B. 2021. Association between clinical respiratory signs, lung lesions detected by thoracic ultrasonography and growth performance in preweaned dairy calves. *Irish Veterinary Journal*, 74, 7.

CUTTANCE, E. L., MASON, W. A., MCDERMOTT, J., LAVEN, R. A., MCDOUGALL, S. & PHYN, C. V. C. 2017. Calf and replacement heifer mortality from birth until weaning in pasture-based dairy herds in New Zealand. *Journal of Dairy Science*, 100, 8347-8357.

DACHRODT, L., ARNDT, H., BARTEL, A., KELLERMANN, L. M., TAUTENHAHN, A., VOLKMANN, M., BIRNSTIEL, K., DO DUC, P., HENTZSCH, A., JENSEN, K. C., KLAWITTER, M., PAUL, P., STOLL, A., WOULDSTRA, S., ZUZ, P., KNUBBEN, G., METZNER, M., MULLER, K. E., MERLE, R. & HOEDEMAKER, M. 2021. Prevalence of disorders in preweaned dairy calves from 731 dairies in Germany: A cross-sectional study. *Journal of Dairy Science*, 104, 9037-9051.

DANTZER, R. 2004. Innate immunity at the forefront of psychoneuroimmunology. *Brain, Behavior, and Immunity*, 18, 1-6.

DANTZER, R. 2023. Evolutionary Aspects of Infections: Inflammation and Sickness Behaviors. In: SAVITZ, J. & YOLKEN, R. H. (eds.) *Microorganisms and Mental Health*. Cham: Springer International Publishing.

DAWKINS, M. S. 2025. Smart farming and Artificial Intelligence (AI): How can we ensure that animal welfare is a priority? *Applied Animal Behaviour Science*, 283, 106519.

DE PAULA VIEIRA, A., DE PASSILLE, A. M. & WEARY, D. M. 2012. Effects of the early social environment on behavioral responses of dairy calves to novel events *Journal of Dairy Science*, 95, 5149-5155.

DE PAULA VIEIRA, A., GUESDON, V., DE PASSILLÉ, A. M., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2008. Behavioural indicators of hunger in dairy calves. *Applied Animal Behaviour Science*, 109, 180-189.

DE PAULA VIEIRA, A., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2010. Effects of pair versus single housing on performance and behavior of dairy calves before and after weaning from milk. *Journal of Dairy Science*, 93, 3079-3085.

- DE VRIES, A. 2020. Symposium review: Why revisit dairy cattle productive lifespan? *Journal of Dairy Science*, 103, 3838-3845.
- DEEPAK, ALY, S. S., LOVE, W. J., BLANCHARD, P. C., CROSSLEY, B., VAN EENENNAAM, A. L. & LEHENBAUER, T. W. 2021. Etiology and risk factors for bovine respiratory disease in pre-weaned calves on California dairies and calf ranches. *Preventive Veterinary Medicine*, 197, 105506.
- DENHOLM, K., EVANS, N. P., BAXTER-SMITH, K. & BURR, P. 2024. Retrospective study of the relative frequency of cattle respiratory disease pathogens in clinical laboratory samples submitted by UK veterinary practices. *Veterinary Record*, 195, e4434.
- DENNIS, T. S., SUAREZ-MENA, F. X., HILL, T. M., QUIGLEY, J. D., SCHLOTTERBECK, R. L., KLOPP, R. N., LASCANO, G. J. & HULBERT, L. 2018. Effects of gradual and later weaning ages when feeding high milk replacer rates on growth, textured starter digestibility, and behavior in Holstein calves from 0 to 4 months of age. *Journal of Dairy Science*, 101, 9863-9875.
- DOMINIAK, K. N. & KRISTENSEN, A. R. 2017. Prioritizing alarms from sensor-based detection models in livestock production - A review on model performance and alarm reducing methods. *Computers and Electronics in Agriculture*, 133, 46-67.
- DONADIO, J. P., DE-SOUSA, K. T., TORRES, R. D. N. S., ALVES, T. C., HÖTZEL, M. J. & DENIZ, M. 2025. A meta-analysis approach to evaluate the effects of early group housing on calf performance, health, and behavior during the preweaning period. *Journal of Dairy Science*, 108, 954-967.
- DONLON, J., MEE, J. F. & MCALOON, C. G. 2025. Association between housing, management and environmental factors and respiratory disease prevalence in dairy calves. *Animal - Science proceedings*, 16, 214-215.
- DONLON, J. D., MCALOON, C. G., HYDE, R., ALY, S., PARDON, B. & MEE, J. F. 2023a. A systematic review of the relationship between housing environmental factors and bovine respiratory disease in preweaned calves - Part 2: Temperature, relative humidity and bedding. *The Veterinary Journal*, 300-302, 106032.
- DONLON, J. D., MCALOON, C. G., HYDE, R., ALY, S., PARDON, B. & MEE, J. F. 2023b. A systematic review of the relationship between housing environmental factors and bovine respiratory disease in preweaned calves – Part 1: Ammonia, air microbial count, particulate matter and endotoxins. *The Veterinary Journal*, 300-302, 106031.
- DONLON, J. D., MCALOON, C. G. & MEE, J. F. 2024. Performance of various interpretations of clinical scoring systems for diagnosis of respiratory disease in dairy calves in a temperate climate using Bayesian latent class analysis. *Journal of Dairy Science*, 107, 7138-7152.

- DONLON, J. D., MEE, J. F. & MCALOON, C. G. 2023c. Prevalence of respiratory disease in Irish preweaned dairy calves using hierarchical Bayesian latent class analysis. *Frontiers in Veterinary Science*, 10.
- DRIESSEN, C. & HEUTINCK, L. F. M. 2015. Cows desiring to be milked? Milking robots and the co-evolution of ethics and technology on Dutch dairy farms. *Agriculture and Human Values*, 32, 3-20.
- DUBROVSKY, S. A., VAN EENENNAAM, A. L., ALY, S. S., KARLE, B. M., ROSSITTO, P. V., OVERTON, M. W., LEHENBAUER, T. W. & FADEL, J. G. 2018. Preweaning cost of bovine respiratory disease (BRD) and cost-benefit of implementation of preventative measures in calves on California dairies: The BRD 10K study. *Journal of Dairy Science*, 103, 1583-1597.
- DUBROVSKY, S. A., VAN EENENNAAM, A. L., KARLE, B. M., ROSSITTO, P. V., LEHENBAUER, T. W. & ALY, S. S. 2019 Bovine respiratory disease (BRD) cause-specific and overall mortality in preweaned calves on California dairies: The BRD 10K study. *Journal of Dairy Science*, 102, 7320-7328.
- DUNN, T. R., OLLIVETT, T. L., RENAUD, D. L., LESLIE, K. E., LEBLANC, S. J., DUFFIELD, T. F. & KELTON, D. F. 2018. The effect of lung consolidation, as determined by ultrasonography, on first-lactation milk production in Holstein dairy calves. *Journal of Dairy Science*, 101, 5404-5410.
- DUTHIE, C. A., BOWEN, J. M., BELL, D. J., MILLER, G. A., MASON, C. & HASKELL, M. J. 2021. Feeding behaviour and activity as early indicators of disease in pre-weaned dairy calves. *Animal*, 15, 100150.
- EASTWOOD, C., AYRE, M., NETTLE, R. & DELA RUE, B. 2019a. Making sense in the cloud: Farm advisory services in a smart farming future. *NJAS: Wageningen Journal of Life Sciences*, 90-91, 1-10.
- EASTWOOD, C., KLERKX, L., AYRE, M. & DELA RUE, B. 2019b. Managing Socio-Ethical Challenges in the Development of Smart Farming: From a Fragmented to a Comprehensive Approach for Responsible Research and Innovation. *Journal of Agricultural and Environmental Ethics*, 32, 741-768.
- EASTWOOD, C. R., JAGO, J. G., EDWARDS, J. P. & BURKE, J. K. 2015. Getting the most out of advanced farm management technologies: roles of technology suppliers and dairy industry organisations in supporting precision dairy farmers. *Animal Production Science*, 56, 1752-1760.
- EASTWOOD, C. R. & RENWICK, A. 2020. Innovation Uncertainty Impacts the Adoption of Smarter Farming Approaches. *Frontiers in Sustainable Food Systems*, Volume 4 - 2020.
- ECKELKAMP, E. A. & BEWLEY, J. M. 2020. On-farm use of disease alerts generated by precision dairy technology. *Journal of Dairy Science*, 103, 1566-1582.

EFSA PANEL ON ANIMAL HEALTH AND ANIMAL WELFARE, NIELSEN, S. S., ALVAREZ, J., BICOUT, D. J., CALISTRI, P., CANALI, E., DREWE, J. A., GARIN-BASTUJI, B., GONZALES ROJAS, J. L., GORTAZAR SCHMIDT, C., HERSKIN, M., MICHEL, V., MIRANDA CHUECA, M. A., PADALINO, B., PASQUALI, P., ROBERTS, H. C., SPOOLDER, H., STAHL, K., VELARDE, A., VILTROP, A., JENSEN, M. B., WAIBLINGER, S., CANDIANI, D., LIMA, E., MOSBACH-SCHULZ, O., VAN DER STEDE, Y., VITALI, M. & WINCKLER, C. 2023. Welfare of calves. *EFSA Journal*, 21, e07896.

EL-DEEB, W., ELSOHABY, I., FAYEZ, M., MKRTCHYAN, H. V., EL-ETRIBY, D. & ELGIOUSHY, M. 2020. Use of procalcitonin, neopterin, haptoglobin, serum amyloid A and proinflammatory cytokines in diagnosis and prognosis of bovine respiratory disease in feedlot calves under field conditions. *Acta Tropica*, 204, 105336.

ELLINGSEN, K., COLEMAN, G. J., LUND, V. & MEJDELL, C. M. 2014. Using qualitative behaviour assessment to explore the link between stockperson behaviour and dairy calf behaviour. *Applied Animal Behaviour Science*, 153, 10-17.

ELLINGSEN, K., COLEMAN, G. J., LUND, V. & MEJDELL, C. M. 2014. Using qualitative behaviour assessment to explore the link between stockperson behaviour and dairy calf behaviour. *Applied Animal Behaviour Science*, 153, 10-17.

ELLINGSEN-DALSKAU, K., DEAN, K. R. & ROUSING, T. 2020. On-farm testing of dairy calves' avoidance response to human approach: Effects of sex, age and test order. *Animal Welfare*, 29, 411-418.

ESSELBURN, K. M., O'DIAM, K. M., HILL, T. M., BATEMAN, H. G., 2ND, ALDRICH, J. M., SCHLOTTERBECK, R. L. & DANIELS, K. M. 2013. Intake of specific fatty acids and fat alters growth, health, and titers following vaccination in dairy calves. *Journal of Dairy Science*, 96, 5826-35.

FAIDAH, N., SORAYA, G. V., ERLICHSTER, M., NATZIR, R., CHANA, G., SKAFIDAS, E., HARDJO, M., GANDA, I. J. & BAHAR, B. 2021. Detection of voluntary dehydration in paediatric populations using non-invasive point-of-care saliva and urine testing. *Journal of Paediatrics and Child Health*, 57, 813-818.

FATHI, E., FARAHZADI, R. & IMANI, M. 2011. Approach to treatment of bronchopneumonia by evaluation of selected acute-phase proteins in calf herds. *Comparative Clinical Pathology*, 22, 125-129.

FERRARI, S., PICCININI, R., SILVA, M., EXADAKTYLOS, V., BERCKMANS, D. & GUARINO, M. 2010. Cough sound description in relation to respiratory diseases in dairy calves. *Preventive Veterinary Medicine*, 96, 276-80.

FISCHER, S., BAUERFEIND, R., CZERNY, C. P. & NEUMANN, S. 2016. Serum interleukin-6 as a prognostic marker in neonatal calf diarrhea. *Journal of Dairy Science*, 99, 6563-6571.

FOWLER, J., STIEGER-VANEGAS, S. M., VANEGAS, J. A., BOBE, G. & POULSEN, K. P. 2017. Comparison of Thoracic Radiography and Computed Tomography in Calves with Naturally Occurring Respiratory Disease. *Frontiers in Veterinary Science*, Volume 4 - 2017.

FUJIWARA, M., HASKELL, M. J., MACRAE, A. I. & RUTHERFORD, K. M. D. 2020. Impact of Maternal High Stocking Density during the Dry Period on Dairy Calf Health, Behaviour, and Welfare. *Animals*, 10.

FUKUDA, T., TSUKANO, K., NAKATSUJI, H. & SUZUKI, K. 2019. Plasma diamine oxidase activity decline with diarrhea severity in calves indicating systemic dysfunction related to intestinal mucosal damage. *Research in Veterinary Science*, 126, 127-130.

FUREIX, C. & MEAGHER, R. K. 2015. What can inactivity (in its various forms) reveal about affective states in non-human animals? A review. *Applied Animal Behaviour Science*, 171, 8-24.

GAILLARD, C., MEAGHER, R. K., VON KEYSERLINGK, M. A. & WEARY, D. M. 2014. Social housing improves dairy calves' performance in two cognitive tests. *PLOS ONE*, 9, e90205.

GINGERICH, K. N., LINDNER, E. E., KALMAN, S. & MILLER-CUSHON, E. K. 2023. Social contact from birth influences personality traits of group-housed dairy calves. *JDS Communications*, 4, 484-488.

GLOVER, I. D., BARRETT, D. C. & REYHER, K. K. 2019. Little association between birth weight and health of preweaned dairy calves. *Veterinary Record*, 184, 477.

GOETZ, H. M., KELTON, D. F., COSTA, J. H. C., WINDER, C. B. & RENAUD, D. L. 2021. Identification of biomarkers measured upon arrival associated with morbidity, mortality, and average daily gain in grain-fed veal calves. *Journal of Dairy Science*, 104, 874-885.

GOH, N., HOUSE, J. & ROWE, S. 2024. Retrospective cohort study investigating the relationship between diarrhea during the preweaning period and subsequent survival, health, and production in dairy cows. *Journal of Dairy Science*, 107, 9752-9761.

GOHARSHAHI, M., AZIZADEH, M., LIDAUER, L., STEININGER, A., KICKINGER, F., OHLSCHUSTER, M., AUER, W., KLEIN-JOBSTL, D., DRILLICH, M. & IWERSEN, M. 2021. Monitoring selected behaviors of calves by use of an ear-attached accelerometer for detecting early indicators of diarrhea. *Journal of Dairy Science*, 104, 6013-6019.

GREEN, C., MACKENZIE, A., GILBERT, D. & ROBINSON, P. A. 2025. Exploring farmers' perceptions of the value and management of dairy-bred calves in block calving dairy systems in England. *Animal - Science proceedings*, 16, 48-49.

- GRIEBEL, P., HILL, K. & STOOKEY, J. 2014. How stress alters immune responses during respiratory infection. *Animal Health Research Reviews*, 15, 161-5.
- GRIEBEL, P. J., SCHOONDERWOERD, M. & BABIUK, L. A. 1987. Ontogeny of the immune response: effect of protein energy malnutrition in neonatal calves. *Canadian Journal of Veterinary Research*, 51, 428-35.
- GRINTER, L. N., CAMPLER, M. R. & COSTA, J. H. C. 2019. Technical note: Validation of a behavior-monitoring collar's precision and accuracy to measure rumination, feeding, and resting time of lactating dairy cows. *Journal of Dairy Science*, 102, 3487-3494.
- GROHER, T., HEITKAMPER, K. & UMSTATTER, C. 2020. Digital technology adoption in livestock production with a special focus on ruminant farming. *Animal*, 14, 2404-2413.
- GULLIKSEN, S. M., LIE, K. I., LOKEN, T. & OSTERAS, O. 2009. Calf mortality in Norwegian dairy herds. *Journal of Dairy Science*, 92, 2782-95.
- GUTIERREZ, A. M., MARTINEZ-SUBIELA, S., ECKERSALL, P. D. & CERON, J. J. 2009. C-reactive protein quantification in porcine saliva: a minimally invasive test for pig health monitoring. *The Veterinary Journal*, 181, 261-5.
- HAMADA, A. M., SABRY, M. H., SAMY, A. & ALANSARY, A. 2013. Salivary Anion Gap and Na/K ratio as Predictive of Acid-Base Balance after Open Heart Surgery. *Life Science Journal-Acta Zhengzhou University Overseas Edition*, 10, 1045-1049.
- HANZLICEK, G. A., WHITE, B. J., MOSIER, D., RENTER, D. G. & ANDERSON, D. E. 2010. Serial evaluation of physiologic, pathological, and behavioral changes related to disease progression of experimentally induced Mannheimia haemolytica pneumonia in postweaned calves. *American Journal of Veterinary Research*, 71, 359-69.
- HART, B. L. 1988. Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral Reviews*, 12, 123-137.
- HAYES, G., MATHEWS, K., KRUTH, S., DOIG, G. & DEWEY, C. 2010. Illness severity scores in veterinary medicine: what can we learn? *Journal of Veterinary Internal Medicine*, 24, 457-66.
- HEINRICHS, A. J. & HEINRICHS, B. S. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *Journal of Dairy Science*, 94, 336-41.
- HILL, T. M., BATEMAN, H. G., 2ND, ALDRICH, J. M., QUIGLEY, J. D. & SCHLOTTERBECK, R. L. 2013. Short communication: intensive measurements of standing time of dairy calves housed in individual pens within a naturally ventilated,

unheated nursery over different periods of the year. *Journal of Dairy Science*, 96, 1811-4.

HELLER, M. C. & CHIGERWE, M. 2018. Diagnosis and Treatment of Infectious Enteritis in Neonatal and Juvenile Ruminants. *Veterinary Clinics of North America: Food Animal Practice*, 34, 101-117.

HENCHION, M. M., REGAN, Á., BEECHER, M. & MACKENWALSH, Á. 2022. Developing 'Smart' Dairy Farming Responsive to Farmers and Consumer-Citizens: A Review. *Animals* [Online], 12.

HILL, T. M., BATEMAN, H. G., 2ND, SUAREZ-MENA, F. X., DENNIS, T. S. & SCHLOTTERBECK, R. L. 2016. Short communication: Changes in body temperature of calves up to 2 months of age as affected by time of day, age, and ambient temperature. *Journal of Dairy Science*, 99, 8867-8870.

HINNANT, H., ELDER, L., CLAUS-WALKER, R., MANDELLA, C., SLANZON, G., PARRISH, L., TROMBETTA, S. & MCCONNELL, C. 2024. Comparative diagnoses of respiratory disease in preweaned dairy calves using sequential thoracic ultrasonography and clinical respiratory scoring. *Australian Veterinary Journal*, 102, 187-199.

HIXSON, C. L., KRAWCZEL, P. D., CALDWELL, J. M. & MILLER-CUSHON, E. K. 2018. Behavioral changes in group-housed dairy calves infected with *Mannheimia haemolytica*. *Journal of Dairy Science*, 101, 10351-10360.

HOFFMANN, G., SCHMIDT, M. & AMMON, C. 2016. First investigations to refine video-based IR thermography as a non-invasive tool to monitor the body temperature of calves. *Animal*, 10, 1542-6.

HOLSTEGE, M. M. C., DE BONT-SMOLENAARS, A. J. G., SANTMAN-BERENDS, I. M. G. A., VAN DER LINDE-WITTEVEEN, G. M., VAN SCHAIK, G., VELTHUIS, A. G. J. & LAM, T. J. G. M. 2018. Factors associated with high antimicrobial use in young calves on Dutch dairy farms: A case-control study. *Journal of Dairy Science*, 101, 9259-9265.

HOPPE, S., BRANDT, H. R., KONIG, S., ERHARDT, G. & GAULY, M. 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. *Journal of Animal Science*, 88, 1982-9.

HORVATH, K. C., ALLEN, A. N. & MILLER-CUSHON, E. K. 2020. Effects of access to stationary brushes and chopped hay on behavior and performance of individually housed dairy calves. *Journal of Dairy Science*, 103, 8421-8432.

HORVATH, K. C. & MILLER-CUSHON, E. K. 2018. Characterizing social behavior, activity, and associations between cognition and behavior upon social grouping of weaned dairy calves. *Journal of Dairy Science*, 101, 7287-7296.

HUANG, J., HARVEY, J., FAM, W. H. D., NIMMO, M. A. & TOK, I. Y. A. 2013. Novel Biosensor for InterLeukin-6 Detection. *Procedia Engineering*, 60, 195-200.

- HULBERT, L. E. & MOISA, S. J. 2016. Stress, immunity, and the management of calves. *Journal of Dairy Science*, 99, 3199-3216.
- HURST, T. S., LOPEZ-VILLALOBOS, N. & BOERMAN, J. P. 2021. Predictive equations for early-life indicators of future body weight in Holstein dairy heifers. *Journal of Dairy Science*, 104, 736-749.
- HURST, T. S., NEVES, R. C. & BOERMAN, J. P. 2022. Early life indicators of first lactation milk yield and the effect of treatment for bovine respiratory disease on survivability and risk of pregnancy in Holstein dairy cattle. *The Veterinary Journal*, 282, 105826.
- HYDE, R. M., GREEN, M. J., SHERWIN, V. E., HUDSON, C., GIBBONS, J., FORSHAW, T., VICKERS, M. & DOWN, P. M. 2020. Quantitative analysis of calf mortality in Great Britain. *Journal of Dairy Science*, 103, 2615-2623.
- IDOATE, I., VANDER LEY, B., SCHULTZ, L. & HELLER, M. 2015. Acute phase proteins in naturally occurring respiratory disease of feedlot cattle. *Veterinary Immunology and Immunopathology*, 163, 221-6.
- ILANGES, A., SHIAO, R., SHAKED, J., LUO, J.-D., YU, X. & FRIEDMAN, J. M. 2022. Brainstem ADCYAP1+ neurons control multiple aspects of sickness behaviour. *Nature*, 609, 761-771.
- JENSEN, M. B. 2004. Computer-Controlled Milk Feeding of Dairy Calves: The Effects of Number of Calves per Feeder and Number of Milk Portions on Use of Feeder and Social Behavior. *Journal of Dairy Science*, 87, 3428-3438.
- JENSEN, M. B., DUVE, L. R. & WEARY, D. M. 2015. Pair housing and enhanced milk allowance increase play behavior and improve performance in dairy calves. *Journal of Dairy Science*, 98, 2568-75.
- JENSEN, M. B. & HOLM, L. 2003. The effect of milk flow rate and milk allowance on feeding related behaviour in dairy calves fed by computer controlled milk feeders. *Applied Animal Behaviour Science*, 82, 87-100.
- JENSEN, M. B., JENSEN, A. & VESTERGAARD, M. 2020. The effect of milk feeding strategy and restriction of meal patterning on behavior, solid feed intake, and growth performance of male dairy calves fed via computer-controlled milk feeders. *Journal of Dairy Science*, 103, 8494-8506.
- JOHNSON, K. F., CHANCELLOR, N., BURN, C. C. & WATHES, D. C. 2017. Prospective cohort study to assess rates of contagious disease in pre-weaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. *Veterinary Record Open*, 4, e000226.

- JOHNSON, K. F., CHANCELLOR, N., BURN, C. C. & WATHES, D. C. 2018. Analysis of pre-weaning feeding policies and other risk factors influencing growth rates in calves on 11 commercial dairy farms. *Animal*, 12, 1413-1423.
- JOHNSON, K. F., CHANCELLOR, N. & WATHES, D. C. 2021. A Cohort Study Risk Factor Analysis for Endemic Disease in Pre-Weaned Dairy Heifer Calves. *Animals*, 11, 1-20.
- JOHNSON, R. W. 2002. The concept of sickness behavior: a brief chronological account of four key discoveries. *Veterinary Immunology and Immunopathology*, 87, 443-450.
- JOHNSTON, D., KENNY, D. A., KELLY, A. K., MCCABE, M. S., MCGEE, M., WATERS, S. M. & EARLEY, B. 2016a. Characterisation of haematological profiles and whole blood relative gene expression levels in Holstein-Friesian and Jersey bull calves undergoing gradual weaning. *Animal*, 10, 1547-56.
- JOHNSTON, D., KENNY, D. A., MCGEE, M., WATERS, S. M., KELLY, A. K. & EARLEY, B. 2016b. Electronic feeding behavioural data as indicators of health status in dairy calves. *Irish Journal of Agricultural and Food Research*, 55, 159-168.
- JONES, G. F., FEENEY, D. A. & MEWS, C. 1998. Comparison of radiographic and necropsy findings of lung lesions in calves after challenge exposure with *Pasteurella multocida*. *American Journal of Veterinary Research*, 59, 1108-1112.
- JONGMAN, E. C. & BUTLER, K. L. 2014. The Effect of Age, Stocking Density and Flooring during Transport on Welfare of Young Dairy Calves in Australia. *Animals*, 4, 184-99.
- JOSHI, V., GUPTA, V. K., BHANUPRAKASH, A. G., MANDAL, R. S. K., DIMRI, U. & AJITH, Y. 2018. Haptoglobin and serum amyloid A as putative biomarker candidates of naturally occurring bovine respiratory disease in dairy calves. *Microbial Pathogenesis*, 116, 33-37.
- JOURQUIN, S., LOWIE, T., DEBRUYNE, F., CHANTILLON, L., VEREECKE, N., BOYEN, F., BOONE, R., BOKMA, J. & PARDON, B. 2023. Dynamics of subclinical pneumonia in male dairy calves in relation to antimicrobial therapy and production outcomes. *Journal of Dairy Science*, 106, 676-689.
- KABU, M., ELITOK, B. & KUCUKKURT, I. 2016. Detection of serum amyloid-A concentration in the calf clinically diagnosed with pneumonia, enteritis and pneumoenteritis. *Ciência Rural*, 46, 293-299.
- KATSOULOS, P. D., KARATZIA, M. A., DEDOUSI, A., CAMO, D. & BOSCO, C. 2020. Milk consumption monitoring as a farmer friendly indicator for advanced treatment in limited fed calves with neonatal diarrhoea syndrome. *Veterinárni Medicína*, 65, 104-110.

- KAYSER, W. C., CARSTENS, G. E., JACKSON, K. S., PINCHAK, W. E., BANERJEE, A. & FU, Y. 2019. Evaluation of statistical process control procedures to monitor feeding behavior patterns and detect onset of bovine respiratory disease in growing bulls. *Journal of Animal Science*, 97, 1158-1170.
- KENT, S., BLUTHÉ, R.-M., KELLEY, K. W. & DANTZER, R. 1992. Sickness behavior as a new target for drug development. *Trends in Pharmacological Sciences*, 13, 24-28.
- KHAN, M. A., WEARY, D. M. & VON KEYSERLINGK, M. A. 2011. Invited review: effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. *Journal of Dairy Science*, 94, 1071-81.
- KIRCHHOFF, J., UHLENBRUCK, S., GORIS, K., KEIL, G. M. & HERRLER, G. 2014. Three viruses of the bovine respiratory disease complex apply different strategies to initiate infection. *Veterinary Research*, 45, 20.
- KLEIN-JOBSTL, D., IWERSEN, M. & DRILLICH, M. 2014. Farm characteristics and calf management practices on dairy farms with and without diarrhea: a case-control study to investigate risk factors for calf diarrhea. *Journal of Dairy Science*, 97, 5110-9.
- KLING-EVEILLARD, F., ALLAIN, C., BOIVIN, X., COURBOULAY, V., CRÉACH, P., PHILIBERT, A., RAMONET, Y. & HOSTIOU, N. 2020. Farmers' representations of the effects of precision livestock farming on human-animal relationships. *Livestock Science*, 238, 104057.
- KMICIKEWYCZ, A. D., DA SILVA, D. N. L., LINN, J. G. & LITHERLAND, N. B. 2013. Effects of milk replacer program fed 2 or 4 times daily on nutrient intake and calf growth. *Journal of Dairy Science*, 96, 1125-1134.
- KNAUER, W. A., GODDEN, S. M., DIETRICH, A., HAWKINS, D. M. & JAMES, R. E. 2018. Evaluation of applying statistical process control techniques to daily average feeding behaviors to detect disease in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, 101, 8135-8145.
- KNAUER, W. A., GODDEN, S. M., DIETRICH, A. & JAMES, R. E. 2017. The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, 100, 5642-5652.
- KNAUER, W. A., GODDEN, S. M., RENDAHL, A. K., ENDRES, M. I. & CROOKER, B. A. 2021. The effect of individual versus pair housing of dairy heifer calves during the preweaning period on measures of health, performance, and behavior up to 16 weeks of age. *Journal of Dairy Science*, 104, 3495-3507.
- KOVACS, L., KEZER, F. L., BAKONY, M., JURKOVICH, V. & SZENCI, O. 2018. Lying down frequency as a discomfort index in heat stressed Holstein bull calves. *Scientific Reports*, 8, 15065.

- KRACHUN, C., RUSHEN, J. & DE PASSILLÉ, A. M. 2010. Play behaviour in dairy calves is reduced by weaning and by a low energy intake. *Applied Animal Behaviour Science*, 122, 71-76.
- KYLE, S. N., DUNNE, S. & BARKUS, E. 2025. Resilience, Loneliness, and Mental Well-Being in the UK Farming Community. *Journal of Agromedicine*, 1-11.
- LAMY, E. & MAU, M. 2012. Saliva proteomics as an emerging, non-invasive tool to study livestock physiology, nutrition and diseases. *Journal of Proteomics*, 75, 4251-8.
- LAUBER, M. C., HEMSWORTH, P. H. & BARNETT, J. L. 2009. The effect of breed, time spent with dam and late pregnancy induction of parturition on behavioural development in dairy calves. *Behavioural Processes*, 82, 301-11.
- LERUSTE, H., BRSCIC, M., COZZI, G., KEMP, B., WOLTHUIS-FILLERUP, M., LENSINK, B. J., BOKKERS, E. A. & VAN REENEN, C. G. 2014. Prevalence and potential influencing factors of non-nutritive oral behaviors of veal calves on commercial farms. *Journal of Dairy Science*, 97, 7021-30.
- LINDLEY, G., BLACKIE, N., WATHES, D. C. & BOOTH, R. E. 2025. Development and Progression of Bovine Respiratory Disease Measured Using Clinical Respiratory Scoring and Thoracic Ultrasonography in Prewaned Calves on Dairy Farms in the United Kingdom: A Prospective Cohort Study. *Animals* [Online], 15.
- LINDLEY, G., BOOTH, R., WATHES, C. & BLACKIE, N. 2024. 56. Inter-rater agreement of different thoracic ultrasonography techniques performed by novice operators on preweaned dairy calves. *Animal - science proceedings*, 15, 62-63.
- LINDNER, E. E., GINGERICH, K. N. & MILLER-CUSHON, E. K. 2021. Effects of early social contact on dairy calf response to initial social grouping and regrouping. *Journal of Dairy Science*, 104, 10090-10099.
- LIU, S., MA, J., LI, J., ALUGONGO, G. M., WU, Z., WANG, Y., LI, S. & CAO, Z. 2019. Effects of Pair Versus Individual Housing on Performance, Health, and Behavior of Dairy Calves. *Animals*, 10.
- LOKHORST, C., DE MOL, R. M. & KAMPHUIS, C. 2019. Invited review: Big Data in precision dairy farming. *Animal*, 13, 1519-1528.
- LOUIE, A. P., ROWE, J. D., LOVE, W. J., LEHENBAUER, T. W. & ALY, S. S. 2018. Effect of the environment on the risk of respiratory disease in preweaning dairy calves during summer months. *Journal of Dairy Science*, 101, 10230-10247.
- LOVE, W. J., LEHENBAUER, T. W., KASS, P. H., VAN EENENNAAM, A. L. & ALY, S. S. 2014. Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. *PeerJ*, 2, e238.

- LOVE, W. J., LEHENBAUER, T. W., VAN EENENNAAM, A. L., DRAKE, C. M., KASS, P. H., FARVER, T. B. & ALY, S. S. 2016. Sensitivity and specificity of on-farm scoring systems and nasal culture to detect bovine respiratory disease complex in preweaned dairy calves. *Journal of Veterinary Diagnostic Investigation*, 28, 119-28.
- LOWE, G., SUTHERLAND, M., WAAS, J., SCHAEFER, A., COX, N. & STEWART, M. 2019a. Infrared Thermography—A Non-Invasive Method of Measuring Respiration Rate in Calves. *Animals* [Online], 9.
- LOWE, G. L., SUTHERLAND, M. A., WAAS, J. R., COX, N. R., SCHAEFER, A. L. & STEWART, M. 2021. Effect of milk allowance on the suitability of automated behavioural and physiological measures as early disease indicators in calves. *Applied Animal Behaviour Science*, 234.
- LOWE, G. L., SUTHERLAND, M. A., WAAS, J. R., SCHAEFER, A. L., COX, N. R. & STEWART, M. 2019b. Physiological and behavioral responses as indicators for early disease detection in dairy calves. *Journal of Dairy Science*, 102, 5389-5402.
- LOWIE, T., JOURQUIN, S., STUYVAERT, S., HANLEY-COOK, G. & PARDON, B. 2025. Associations between lung consolidation and serum amyloid A and haptoglobin, and the potential of acute phase proteins to differentiate primary respiratory tract pathogens in calves. *Journal of Dairy Science*, 108, 943-953.
- LOWIE, T., VAN LEENEN, K., JOURQUIN, S., PAS, M. L., BOKMA, J. & PARDON, B. 2022. Differences in the association of cough and other clinical signs with ultrasonographic lung consolidation in dairy, veal, and beef calves. *Journal of Dairy Science*, 105, 6111-6124.
- LOWIE, T., VANDEWALLE, J., HANLEY-COOK, G., PARDON, B. & BOKMA, J. 2024. Short communication: Circadian variations and day-to-day variability of clinical signs used for the early diagnosis of pneumonia within and between calves. *Research in Veterinary Science*, 166, 105082.
- LU, Y. P., HUANG, J. W., LEE, I. N., WENG, R. C., LIN, M. Y., YANG, J. T. & LIN, C. T. 2019. A Portable System to Monitor Saliva Conductivity for Dehydration Diagnosis and Kidney Healthcare. *Scientific Reports*, 9, 14771.
- LUBBERS, B. V., APLEY, M. D., COETZEE, J. F., MOSIER, D. A., BILLER, D. S., MASON, D. E. & HENAO-GUERRERO, P. N. 2007. Use of computed tomography to evaluate pathologic changes in the lungs of calves with experimentally induced respiratory tract disease. *American Journal of Veterinary Research*, 68, 1259-1264.
- LÜRZEL, S., MÜNSCH, C., WINDSCHNURER, I., FUTSCHIK, A., PALME, R. & WAIBLINGER, S. 2015. The influence of gentle interactions on avoidance distance towards humans, weight gain and physiological parameters in group-housed dairy calves. *Applied Animal Behaviour Science*, 172, 9-16.

LV, J., ZHAO, X.-W., SU, H., WANG, Z.-P., WANG, C., LI, J.-H., LI, X., ZHANG, R.-X. & BAO, J. 2021. Effects of group size on the behaviour, heart rate, immunity, and growth of Holstein dairy calves. *Applied Animal Behaviour Science*, 241.

MACHADO, V. S. & BALLOU, M. A. 2022. Overview of common practices in calf raising facilities. *Translational Animal Science*, 6, txab234.

MAHENDRAN, S. A. 2020. Use of fever detection in combination with thoracic ultrasonography to identify respiratory disease, and compare treatments of antimicrobials and NSAID: a randomised study in dairy calves. *Veterinary Record Open*, 7, e000415.

MAHENDRAN, S. A., BOOTH, R., BEEKHUIS, L., MANNING, A., BLACKMORE, T., VANHOUDT, A. & BELL, N. 2017. Assessing the effects of weekly preweaning health scores on dairy calf mortality and productivity parameters: cohort study. *Veterinary Record*, 181, 196.

MAHENDRAN, S. A., WATHES, D. C., BOOTH, R. E. & BLACKIE, N. 2022. A survey of calf management practices and farmer perceptions of calf housing in UK dairy herds. *Journal of Dairy Science*, 105, 409-423.

MANDEL, R., WHAY, H. R., KLEMENT, E. & NICOL, C. J. 2016. Invited review: Environmental enrichment of dairy cows and calves in indoor housing. *Journal of Dairy Science*, 99, 1695-1715.

MASSET, N., ASSIÉ, S., HERMAN, N., JOZAN, T. & HERRY, V. 2022. Ultrasonography of the cranial part of the thorax is a quick and sensitive technique to detect lung consolidation in veal calves. *Veterinary Medicine and Science*, 8, 1229-1239.

MARTIN, M., KLEINHENZ, M. D., MONTGOMERY, S. R., BLASI, D. A., ALMES, K. M., BAYSINGER, A. K. & COETZEE, J. F. 2022. Assessment of diagnostic accuracy of biomarkers to assess lung consolidation in calves with induced bacterial pneumonia using receiver operating characteristic curves. *Journal of Animal Science*, 100.

MATTACHINI, G., RIVA, E. & PROVOLO, G. 2011. The lying and standing activity indices of dairy cows in free-stall housing. *Applied Animal Behaviour Science*, 129, 18-27.

MAURER, D., KOZIEL, J., ENGELKEN, T., COOPER, V. & FUNK, J. 2018. Detection of Volatile Compounds Emitted from Nasal Secretions and Serum: Towards Non-Invasive Identification of Diseased Cattle Biomarkers. *Separations*, 5.

MCCONNEL, C. S., NELSON, D. D., BURBICK, C. R., BUHRIG, S. M., WILSON, E. A., KLATT, C. T. & MOORE, D. A. 2019. Clarifying dairy calf mortality phenotypes through postmortem analysis. *Journal of Dairy Science*, 102, 4415-4426.

MCCORKELL, R., WYNNE-EDWARDS, K., WINDEYER, C., SCHAEFER, A. & OF, U. C. 2014. Limited efficacy of Fever Tag temperature sensing ear tags in calves with

naturally occurring bovine respiratory disease or induced bovine viral diarrhoea virus infection. *Canadian Veterinary Journal*, 55, 688-90.

MCGILL, J. L., RUSK, R. A., GUERRA-MAUPOME, M., BRIGGS, R. E. & SACCO, R. E. 2016. Bovine Gamma Delta T Cells Contribute to Exacerbated IL-17 Production in Response to Co-Infection with Bovine RSV and Mannheimia haemolytica. *PLOS ONE*, 11, e0151083.

MCGUIRK, S. M. 2008. Disease management of dairy calves and heifers. *Veterinary Clinics of North America: Food Animal Practice*, 24, 139-53.

MCGUIRK, S. M. & PEEK, S. F. 2014. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. *Animal Health Research Reviews*, 15, 145-7.

MEAGHER, R. K., DAROS, R. R., COSTA, J. H., VON KEYSERLINGK, M. A., HOTZEL, M. J. & WEARY, D. M. 2015. Effects of Degree and Timing of Social Housing on Reversal Learning and Response to Novel Objects in Dairy Calves. *PLOS ONE*, 10, e0132828.

MEDRANO-GALARZA, C., LEBLANC, S. J., DEVRIES, T. J., JONES-BITTON, A., RUSHEN, J., MARIE DE PASSILLÉ, A. & HALEY, D. B. 2017. A survey of dairy calf management practices among farms using manual and automated milk feeding systems in Canada. *Journal of Dairy Science*, 100, 6872-6884.

MEDRANO-GALARZA, C., LEBLANC, S. J., JONES-BITTON, A., DEVRIES, T. J., RUSHEN, J., MARIE DE PASSILLE, A., ENDRES, M. I. & HALEY, D. B. 2018. Associations between management practices and within-pen prevalence of calf diarrhoea and respiratory disease on dairy farms using automated milk feeders. *Journal of Dairy Science*, 101, 2293-2308.

MELCHNER, A., VAN DE BERG, S., SCUDA, N., FEUERSTEIN, A., HANCZARUK, M., SCHUMACHER, M., STRAUBINGER, R. K., MAROSEVIC, D. & RIEHM, J. M. 2021. Antimicrobial Resistance in Isolates from Cattle with Bovine Respiratory Disease in Bavaria, Germany. *Antibiotics*, 10.

MELLOR, D. J. & STAFFORD, K. J. 2004. Animal welfare implications of neonatal mortality and morbidity in farm animals. *The Veterinary Journal*, 168, 118-133.

MERTENS, K., DECUYPERE, E., DE BAERDEMAEKER, J. & DE KETELAERE, B. 2010. Statistical control charts as a support tool for the management of livestock production. *The Journal of Agricultural Science*, 149, 369-384.

MILLMAN, S. T. 2007. Sickness behaviour and its relevance to animal welfare assessment at the group level. *Animal Welfare*, 16, 123-125.

MOHD NOR, N., STEENEVELD, W., MOURITS, M. C. & HOGVEEN, H. 2012. Estimating the costs of rearing young dairy cattle in the Netherlands using a

simulation model that accounts for uncertainty related to diseases. *Preventative Veterinary Medicine*, 106, 214-24.

MOISA, S. J., ALY, S. S., LEHENBAUER, T. W., LOVE, W. J., ROSSITTO, P. V., VAN EENENNAAM, A. L., TROMBETTA, S. C., BORTOLUZZI, E. M. & HULBERT, L. E. 2019. Association of plasma haptoglobin concentration and other biomarkers with bovine respiratory disease status in pre-weaned dairy calves. *Journal of Veterinary Diagnostic Investigation*, 31, 40-46.

MONTES, M. E., DOUCETTE, J., BRITO, L. F. & BOERMAN, J. P. 2023. Environmental and biological factors that influence feeding behavior of Holstein calves in automated milk feeding systems. *JDS Communications*, 4, 379-384.

MORRISON, J., RENAUD, D. L., CHURCHILL, K. J., COSTA, J. H. C., STEELE, M. A. & WINDER, C. B. 2021. Predicting morbidity and mortality using automated milk feeders: A scoping review. *Journal of Dairy Science*, 104, 7177-7194.

MORRISON, J. L., WINDER, C. B., MEDRANO-GALARZA, C., DENIS, P., HALEY, D., LEBLANC, S. J., COSTA, J., STEELE, M. & RENAUD, D. L. 2022. Case-control study of behavior data from automated milk feeders in healthy or diseased dairy calves. *JDS Communications*, 3, 201-206.

MUNOZ, C. X., JOHNSON, E. C., DEMARTINI, J. K., HUGGINS, R. A., MCKENZIE, A. L., CASA, D. J., MARESH, C. M. & ARMSTRONG, L. E. 2013. Assessment of hydration biomarkers including salivary osmolality during passive and active dehydration. *European Journal of Clinical Nutrition*, 67, 1257-63.

MURRAY, C. F., WINDEYER, M. C., DUFFIELD, T. F., HALEY, D. B., PEARL, D. L., WAALDERBOS, K. M. & LESLIE, K. E. 2014. Associations of serum haptoglobin in newborn dairy calves with health, growth, and mortality up to 4 months of age. *Journal of Dairy Science*, 97, 7844-55.

MURRAY, G. M., CASSIDY, J. P., CLEGG, T. A., TRATALOS, J. A., MCCLURE, J., O'NEILL, R. G., SAMMIN, D. J., CASEY, M. J., MCELROY, M., EARLEY, B., BOURKE, N. & MORE, S. J. 2016. A retrospective epidemiological analysis of risk factors for a primary necropsy diagnosis of bovine respiratory disease. *Preventive Veterinary Medicine*, 132, 49-56.

NEETHIRAJAN, S. 2023. The Significance and Ethics of Digital Livestock Farming. *AgriEngineering* [Online], 5.

NEJAD, J. G., HOSSEINDOUST, A., SHOAE, A., GHORBANI, B., LEE, B. H., OSKOUEIAN, E., HAJILARI, D., AMOUZMEHR, A., LOHAKARE, J. D. & SUNG, K. I. 2013. Effects of feeding levels of starter on weaning age, performance, nutrient digestibility and health parameters in holstein dairy calves. *Asian-Australasian Journal of Animal Sciences*, 26, 827-30.

- OCCHIUTO, F., VÁZQUEZ-DIOSDADO, J. A., THOMAS, M., GAYNER, E. R., KING, A. J. & KALER, J. 2025. Dairy calves provided with environmental enrichment are more active, playful and have fewer feeding interruptions. *Scientific Reports*, 15, 4241.
- OIKONOMOU, G., TEIXEIRA, A. G., FODITSCH, C., BICALHO, M. L., MACHADO, V. S. & BICALHO, R. C. 2013. Fecal microbial diversity in pre-weaned dairy calves as described by pyrosequencing of metagenomic 16S rDNA. Associations of Faecalibacterium species with health and growth. *PLOS ONE*, 8, e63157.
- OK, M., YILDIZ, R., HATIPOGLU, F., BASPINAR, N., IDER, M., UNEY, K., ERTURK, A., DURGUT, M. K. & TERZI, F. 2020. Use of intestine-related biomarkers for detecting intestinal epithelial damage in neonatal calves with diarrhea. *American Journal of Veterinary Research*, 81, 139-146.
- OLAGUNJU, L. K., CASPER, D. P. & ANELE, U. Y. 2024. Growth performance of neonatal calves fed milk replacer 2 vs. 3 times per day. *Translational Animal Science*, 8, txae151.
- OLIVEIRA, V. H. S., DALL AGNOL, A. M., FRITZEN, J. T. T., LORENZETTI, E., ALFIERI, A. A. & ALFIERI, A. F. 2020. Microbial diversity involved in the etiology of a bovine respiratory disease outbreak in a dairy calf rearing unit. *Comparative Immunology, Microbiology and Infectious Diseases*, 71, 101494.
- OLIVERA, M. T., MELLADO, J., GARCÍA, J. E., ENCINA, J. A., ÁLVAREZ, P., MACÍAS-CRUZ, U., AVENDAÑO, L. & MELLADO, M. 2025a. The effect of failed transfer of passive immunity and agammaglobulinemia on the occurrence of preweaning diarrhea, pneumonia, and mortality in Holstein calves: English. *Spanish Journal of Agricultural Research*, 22, 20913.
- OLIVERA, M. T., MELLADO, J., GARCÍA, J. E., ENCINA, J. A., ÁLVAREZ, P., MACÍAS-CRUZ, U., AVENDAÑO, L. & MELLADO, M. 2025b. The influence of calfhood diarrhea and pneumonia on preweaning growth and reproductive performance of Holstein heifers: Calfhood diseases and fertility in Holstein heifers. *Spanish Journal of Agricultural Research*, 22, 21154.
- OLLIVETT, T. L. & BUCZINSKI, S. 2016. On-Farm Use of Ultrasonography for Bovine Respiratory Disease. *Veterinary Clinics of North America: Food Animal Practice*, 32, 19-35.
- OLLIVETT, T. L., CASWELL, J. L., NYDAM, D. V., DUFFIELD, T., LESLIE, K. E., HEWSON, J. & KELTON, D. 2015. Thoracic Ultrasonography and Bronchoalveolar Lavage Fluid Analysis in Holstein Calves with Subclinical Lung Lesions. *Journal of Veterinary Internal Medicine*, 29, 1728-34.
- OLSON, A., SISCHO, W. M., BERGE, A. C. B., ADAMS-PROGAR, A. & MOORE, D. A. 2019. A retrospective cohort study comparing dairy calf treatment decisions by

farm personnel with veterinary observations of clinical signs. *Journal of Dairy Science*, 102, 6391-6403.

ORRO, T., POHJANVIRTA, T., RIKULA, U., HUOVILAINEN, A., ALASUUTARI, S., SIHVONEN, L., PELKONEN, S. & SOVERI, T. 2011. Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus. *Comparative Immunology, Microbiology and Infectious Diseases*, 34, 23-9.

OVERVEST, M. A., CROSSLEY, R. E., MILLER-CUSHON, E. K. & DEVRIES, T. J. 2018. Social housing influences the behavior and feed intake of dairy calves during weaning. *Journal of Dairy Science*, 101, 8123-8134.

PARDON, B., CALLENS, J., MARIS, J., ALLAIS, L., VAN PRAET, W., DEPREZ, P. & RIBBENS, S. 2020. Pathogen-specific risk factors in acute outbreaks of respiratory disease in calves. *Journal of Dairy Science*, 103, 2556-2566.

PAUDYAL, S., MAUNSELL, F., RICHESON, J., RISCO, C., DONOVAN, A. & PINEDO, P. 2016. Peripartal rumination dynamics and health status in cows calving in hot and cool seasons. *Journal of Dairy Science*, 99, 9057-9068.

PEMPEK, J. A., EASTRIDGE, M. L. & PROUDFOOT, K. L. 2017. The effect of a furnished individual hutch pre-weaning on calf behavior, response to novelty, and growth. *Journal of Dairy Science*, 100, 4807-4817.

PEMPEK, J. A., WATKINS, L. R., BRUNER, C. E. & HABING, G. G. 2019. A multisite, randomized field trial to evaluate the influence of lactoferrin on the morbidity and mortality of dairy calves with diarrhea. *Journal of Dairy Science*, 102, 9259-9267.

PENATI, M., SALA, G., BISCARINI, F., BOCCARDO, A., BRONZO, V., CASTIGLIONI, B., CREMONESI, P., MORONI, P., PRAVETTONI, D. & ADDIS, M. F. 2021. Feeding Pre-weaned Calves With Waste Milk Containing Antibiotic Residues Is Related to a Higher Incidence of Diarrhea and Alterations in the Fecal Microbiota. *Frontiers in Veterinary Science*, Volume 8 - 2021.

PERRY, V. E. A., COPPING, K. J., MIGUEL-PACHECO, G. & HERNANDEZ-MEDRANO, J. 2019. The Effects of Developmental Programming upon Neonatal Mortality. *Veterinary Clinics of North America: Food Animal Practice*, 35, 289-302.

PORTER, M. M., MCDONALD, P. O., SLATE, J. R., KREUDER, A. J. & MCGILL, J. L. 2021. Use of Thoracic Ultrasonography to Improve Disease Detection in Experimental BRD Infection. *Frontiers in Veterinary Science*, 8, 763972.

PRINCISVAL, L., BOCCARDO, A., PRAVETTONI, D., FERRARO, S., VALARCHER, J.-F., GOMES, V., FECTEAU, G. & BUCZINSKI, S. 2025. Agreement of Specific Lung Sounds Auscultation by Veterinarians for the Detection of Bronchopneumonia in Calves. *Journal of Veterinary Internal Medicine*, 39, e70203.

- QUIGLEY, J. D., WOLFE, T. A. & ELSASSER, T. H. 2006. Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites in calves. *Journal of Dairy Science*, 89, 207-16.
- QUIMBY, W. F., SOWELL, B. F., BOWMAN, J. G. P., BRANINE, M. E., HUBBERT, M. E. & SHERWOOD, H. W. 2001. Application of feeding behaviour to predict morbidity of newly received calves in a commercial feedlot. *Canadian Journal of Animal Science*, 81, 315-320.
- RABOISSON, D., DELOR, F., CAHUZAC, E., GENDRE, C., SANS, P. & ALLAIRE, G. 2013. Perinatal, neonatal, and rearing period mortality of dairy calves and replacement heifers in France. *Journal of Dairy Science*, 96, 2913-24.
- RAMEZANI GARDALOU, N., GUSE, C., LIDAUER, L., STEININGER, A., KICKINGER, F., ÖHLSCHUSTER, M., AUER, W., IWERSEN, M., DRILLICH, M. & KLEIN-JÖBSTL, D. 2022. Early Detection of Respiratory Diseases in Calves by Use of an Ear-Attached Accelerometer. *Animals* [Online], 12.
- RAMOS, J., VILLETAZ-ROBICHAUD, M., DUBUC, J., SANTSCHI, D., ROY, J.-P., FECTEAU, G. & BUCZINSKI, S. 2025. Graduate Student Literature Review: Integrating concepts of animal welfare and health-related quality of life for preweaning dairy calves. *Journal of Dairy Science*, 108, 3868-3877.
- RAOOFI, A., DOOZ, M. G. & HASANLU, J. 2009. The pupillary light reflex and menace response in neonatal calves: The role of environmental isolation on development of the menace response. *Veterinary Journal*, 181, 296-298.
- RATTANAPANADDA, P., RAMSAY, D., BUTTERS, A., BOOKER, C. W., HANNON, S. J., HENDRICK, S., VAN DONKERSGOED, J., WARR, B. N., GOW, S. P. & MORLEY, P. S. 2025. The prevalence and antimicrobial resistance of respiratory pathogens isolated from feedlot cattle in Canada. *Frontiers in Microbiology*, 16.
- REIPURTH, M., KLAUSEN, S. K., DENWOOD, M., FORKMAN, B. & HOUE, H. 2020. The effect of age when group housed and other management factors on playing and non-nutritive sucking behaviour in dairy calves: a cross sectional observational study. *Acta Veterinaria Scandinavica*, 62, 63.
- REITEN, M., ROUSING, T., THOMSEN, P. T., OTTEN, N. D., FORKMAN, B., HOUE, H., SORENSEN, J. T. & KIRCHNER, M. K. 2018. Mortality, diarrhea and respiratory disease in Danish dairy heifer calves: Effect of production system and season. *Preventive Veterinary Medicine*, 155, 21-26.
- RESPONSIBLE USE OF MEDINES IN AGRICULTURE ALLIANCE 2020. Targets Task Force Report 2020: Responsible use of antibiotics in UK farming, Progress against 2020 targets, New targets 2021-2024. <https://ruma.org.uk/wp-content/uploads/2022/07/RUMA-REPORT-021220.pdf>.

RESPONSIBLE USE OF MEDINES IN AGRICULTURE ALLIANCE 2024. RUMA Targets Task Force 2: Four Years On. <https://www.ruma.org.uk/wp-content/uploads/2024/11/RUMA-TTF-Report-FINAL-published-November-19-2024.pdf>.

RHODES, V., RYAN, E. G., HAYES, C. J., MCALOON, C., O'GRADY, L., HOEY, S., MEE, J. F., PARDON, B., EARLEY, B. & MCALOON, C. G. 2021. Diagnosis of respiratory disease in preweaned dairy calves using sequential thoracic ultrasonography and clinical respiratory scoring: Temporal transitions and association with growth rates. *Journal of Dairy Science*, 104, 11165-11175.

RICHESON, J. T. 2020. Behavior assessment and applications for BRD diagnosis: beef. *Animal Health Research Reviews*, 21, 192-195.

RIOJA-LANG, F. C., CONNOR, M., BACON, H. J., LAWRENCE, A. B. & DWYER, C. M. 2020. Prioritization of Farm Animal Welfare Issues Using Expert Consensus. *Frontiers in Veterinary Science*, 6.

ROBERT, B., WHITE, B. J., RENTER, D. G. & LARSON, R. L. 2009. Evaluation of three-dimensional accelerometers to monitor and classify behavior patterns in cattle. *Computers and Electronics in Agriculture*, 67, 80-84.

ROCHE, S. M., GENORE, R., RENAUD, D. L., SHOCK, D. A., BAUMAN, C., CROYLE, S., BARKEMA, H. W., DUBUC, J., KEEFE, G. P. & KELTON, D. F. 2020. Short communication: Describing mortality and euthanasia practices on Canadian dairy farms. *Journal of Dairy Science*, 103, 3599-3605.

RODRIGUEZ, F., GONZALEZ, J. F., ARBELO, M., ZUCCA, D. & FERNANDEZ, A. 2015. Cytokine expression in lungs of calves spontaneously infected with *Mycoplasma bovis*. *Veterinary Research Communications*, 39, 69-72.

ROSE, D. C., MORRIS, C., LOBLEY, M., WINTER, M., SUTHERLAND, W. J. & DICKS, L. V. 2018. Exploring the spatialities of technological and user re-scripting: The case of decision support tools in UK agriculture. *Geoforum*, 89, 11-18.

RUMINANT HEALTH & WELFARE GROUP 2025. GB Dairy Calf Strategy: A review of progress 2020-2023. <https://ruminanthw.org.uk/wp-content/uploads/2025/01/GB-dairy-calf-strategy-review-of-progress-Jan-2025-APP-compressed.pdf>.

SÁADATNIA, A., MOHAMMADI, G. R., AZIZZADEH, M., MIRSHAHI, A., MOHIEDDINI, A. A. & BUCZINSKI, S. 2023. Effect of ultrasonographic lung consolidation on health and growth in dairy calves: A longitudinal study. *Journal of Dairy Science*, 106, 8047-8059.

SALA, G., BOCCARDO, A., FERRULLI, V., MEUCCI, V., DE MARCHI, L., SGOBINI, M., CASTELLI, M., PRAVETTONI, D. & BONELLI, F. 2024. Cross-sectional study: can endogenous procalcitonin differentiate between healthy and bovine respiratory disease-affected preweaned dairy calves? *Veterinary Quarterly*, 44, 1-10.

- SANTOS, M. T. B., BATISTA, R., GUARÉ, R. O., LEITE, M. F., FERREIRA, M. C. D., DURÃO, M. S., NASCIMENTO, O. A. & JARDIM, J. R. 2011. Salivary osmolality and hydration status in children with cerebral palsy. *Journal of Oral Pathology & Medicine*, 40, 582-586.
- SAYAR, E. & KELES, İ. 2024. Investigation of the diagnostic and prognostic importance of Tumor Necrosis Factor- α (TNF- α), Procalcitonin (PCT), Interleukin-6 (IL-6) and Haptoglobin (HP) in calves with neonatal diarrhea. *Veterinary Immunology and Immunopathology*, 277, 110837.
- SCHAEFER, A. L., COOK, N. J., BENCH, C., CHABOT, J. B., COLYN, J., LIU, T., OKINE, E. K., STEWART, M. & WEBSTER, J. R. 2012. The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography. *Research in Veterinary Science*, 93, 928-35.
- SCHAEFER, A. L., COOK, N. J., CHURCH, J. S., BASARAB, J., PERRY, B., MILLER, C. & TONG, A. K. 2007. The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. *Research in Veterinary Science*, 83, 376-84.
- SCHAFFER, A. P., LARSON, R. L., CERNICCHIARO, N., HANZLICEK, G. A., BARTLE, S. J. & THOMSON, D. U. 2016. The association between calfhood bovine respiratory disease complex and subsequent departure from the herd, milk production, and reproduction in dairy cattle. *Journal of the American Veterinary Medical Association*, 248, 1157-64.
- SCHILLINGS, J., BENNETT, R. & ROSE, D. C. 2021. Exploring the Potential of Precision Livestock Farming Technologies to Help Address Farm Animal Welfare. *Frontiers in Animal Science*, Volume 2 - 2021.
- SCHINWALD, M., CREUTZINGER, K., KEUNEN, A., WINDER, C. B., HALEY, D. & RENAUD, D. L. 2022. Predictors of diarrhea, mortality, and weight gain in male dairy calves. *Journal of Dairy Science*, 105, 5296-5309.
- SCOLEY, G., GORDON, A. & MORRISON, S. 2019. Using Non-Invasive Monitoring Technologies to Capture Behavioural, Physiological and Health Responses of Dairy Calves to Different Nutritional Regimes during the First Ten Weeks of Life. *Animals*, 9.
- SCULLY, S., EARLEY, B., SMITH, P. E., MCALOON, C. & WATERS, S. M. 2024. Health-associated changes of the fecal microbiota in dairy heifer calves during the pre-weaning period. *Frontiers in Microbiology*, 15.
- SHIMBO, G., TAGAWA, M., MATSUMOTO, K., TOMIHARI, M., YANAGAWA, M., UEDA, Y., INOKUMA, H. & MIYAHARA, K. 2019. Three-legged radiographic view for evaluating cranioventral lung region in standing calves with bovine respiratory disease. *Journal of Veterinary Medical Science*, 81, 120-126.

- SHIP, J. A. & FISCHER, D. J. 1997. The relationship between dehydration and parotid salivary gland function in young and older healthy adults. *The Journals of Gerontology: Series A*, 52, M310-9.
- SHIVLEY, C. B., LOMBARD, J. E., URIE, N. J., KOPRAL, C. A., SANTIN, M., EARLEYWINE, T. J., OLSON, J. D. & GARRY, F. B. 2018. Preweaned heifer management on US dairy operations: Part VI. Factors associated with average daily gain in preweaned dairy heifer calves. *Journal of Dairy Science*, 101, 9245-9258.
- SHU, H., BINDELLE, J. & GU, X. 2024. Non-contact respiration rate measurement of multiple cows in a free-stall barn using computer vision methods. *Computers and Electronics in Agriculture*, 218.
- SINNOTT, A. M., KENNEDY, E. & BOKKERS, E. A. M. 2021. The effects of manual and automated milk feeding methods on group-housed calf health, behaviour, growth and labour. *Livestock Science*, 244.
- SMITH, D. L., SHALMIYEVA, I., DEBLOIS, J. & WINKE, M. 2012. Use of salivary osmolality to assess dehydration. *Prehospital Emergency Care*, 16, 128-35.
- SMITH, G. W. & BERCHTOLD, J. 2014. Fluid therapy in calves. *Veterinary Clinics of North America: Food Animal Practice*, 30, 409-27, vi.
- ŠOLTÉSOVÁ, H., NAGY, O., TÓTHOVÁ, C., PAULÍKOVÁ, I. & SEIDEL, H. 2015. Blood Gases, Acid-Base Status And Plasma Lactate Concentrations In Calves With Respiratory Diseases. *Acta Veterinaria*, 65, 111-124.
- SONNTAG, N., BORCHARDT, S., HEUWIESER, W. & SUTTER, F. 2024. Association between a pyroelectric infrared sensor monitoring system and a 3-dimensional accelerometer to assess movement in preweaning dairy calves. *JDS Communications*, 5, 72-76.
- SRUC VETERINARY SERVICES 2019. Mortality in preweaning dairy calves. *Veterinary Record*, 185, 723-723.
- STANTON, A. L., KELTON, D. F., LEBLANC, S. J., WORMUTH, J. & LESLIE, K. E. 2012. The effect of respiratory disease and a preventative antibiotic treatment on growth, survival, age at first calving, and milk production of dairy heifers. *Journal of Dairy Science*, 95, 4950-4960.
- STEENSELS, M., BAHR, C., BERCKMANS, D., HALACHMI, I., ANTLER, A. & MALTZ, E. 2012. Lying patterns of high producing healthy dairy cows after calving in commercial herds as affected by age, environmental conditions and production. *Applied Animal Behaviour Science*, 136, 88-95.
- STUDDS, M. J., DEIKUN, L. L., SORTER, D. E., PEMPEK, J. A. & PROUDFOOT, K. L. 2018. Short communication: The effect of diarrhea and navel inflammation on the lying behavior of veal calves. *Journal of Dairy Science*, 101, 11251-11255.

- SUN, D., WEBB, L., VAN DER TOL, P. P. J. & VAN REENEN, K. 2021. A Systematic Review of Automatic Health Monitoring in Calves: Glimpsing the Future From Current Practice. *Frontiers in Veterinary Science*, 8, 761468.
- SUTHERLAND, M. A., LOWE, G. L., HUDDART, F. J., WAAS, J. R. & STEWART, M. 2018. Measurement of dairy calf behavior prior to onset of clinical disease and in response to disbudding using automated calf feeders and accelerometers. *Journal of Dairy Science*, 101, 8208-8216.
- SUTHERLAND, M. A., WORTH, G. M., CAMERON, C., ROSS, C. M. & RAPP, D. 2017. Health, physiology, and behavior of dairy calves reared on 4 different substrates. *Journal of Dairy Science*, 100, 2148-2156.
- SUTHERLAND, M. A., WORTH, G. M., SCHÜTZ, K. E. & STEWART, M. 2014. Rearing substrate and space allowance influences locomotor play behaviour of dairy calves in an arena test. *Applied Animal Behaviour Science*, 154, 8-14.
- SUZUKI, D., YAMADA, S. I., SAKURAI, A., KARASAWA, I., KONDO, E., SAKAI, H., TANAKA, H., SHIMANE, T. & KURITA, H. 2020. Correlations between the properties of saliva and metabolic syndrome: A prospective observational study. *Medicine (Baltimore)*, 99, e23688.
- SVENSSON, C. & JENSEN, M. B. 2007. Short Communication: Identification of Diseased Calves by Use of Data from Automatic Milk Feeders. *Journal of Dairy Science*, 90, 994-997.
- SWARTZ, T. H., FINDLAY, A. N. & PETERSSON-WOLFE, C. S. 2017. Short communication: Automated detection of behavioral changes from respiratory disease in pre-weaned calves. *Journal of Dairy Science*, 100, 9273-9278.
- SWARTZ, T. H., SCHRAMM, H. H. & PETERSSON-WOLFE, C. S. 2020. Short Communication: Association between neonatal calf diarrhea and lying behaviors. *Veterinary and Animal Science*, 9, 100111.
- SYLVIA, K. E. & DEMAS, G. E. 2017. A Return to Wisdom: Using Sickness Behaviors to Integrate Ecological and Translational Research. *Integrative and Comparative Biology*, 57, 1204-1213.
- SZYSZKA, O. & KYRIAZAKIS, I. 2013. What is the relationship between level of infection and 'sickness behaviour' in cattle? *Applied Animal Behaviour Science*, 147, 1-10.
- TAYLOR, N. A., VAN DEN HEUVEL, A. M., KERRY, P., MCGHEE, S., PEOPLES, G. E., BROWN, M. A. & PATTERSON, M. J. 2012. Observations on saliva osmolality during progressive dehydration and partial rehydration. *European Journal of Applied Physiology*, 112, 3227-37.

- TAYLOR-HOLT, E. N., KOSSAIBATI, M. A., CHANNA, K., HANKS, J. & TAYLOR, N. M. 2025. Milk recording data highlight improvements in fertility and somatic cell counts but worsening longevity for the UK national dairy herd. *Livestock Science*, 298, 105739.
- TEIXEIRA, A. G. V., MCART, J. A. A. & BICALHO, R. C. 2017. Thoracic ultrasound assessment of lung consolidation at weaning in Holstein dairy heifers: Reproductive performance and survival. *Journal of Dairy Science*, 100, 2985-2991.
- THEURER, M. E., WHITE, B. J., LARSON, R. L. & SCHROEDER, T. C. 2015. A stochastic model to determine the economic value of changing diagnostic test characteristics for identification of cattle for treatment of bovine respiratory disease. *Journal of Animal Science*, 93, 1398-410.
- THORUP, V., SAINI, D., SCOLEY, G. & MORRISON, S. 2021. 153 Are male calves lazier than female calves? *Animal - science proceedings*, 12, 123.
- THORUP, V. M., MUNKSGAARD, L., ROBERT, P. E., ERHARD, H. W., THOMSEN, P. T. & FRIGGENS, N. C. 2015. Lameness detection via leg-mounted accelerometers on dairy cows on four commercial farms. *Animal*, 9, 1704-12.
- TIMSIT, E., DENDUKURI, N., SCHILLER, I. & BUCZINSKI, S. 2016. Diagnostic accuracy of clinical illness for bovine respiratory disease (BRD) diagnosis in beef cattle placed in feedlots: A systematic literature review and hierarchical Bayesian latent-class meta-analysis. *Preventive Veterinary Medicine*, 135, 67-73.
- TOAFF-ROSENSTEIN, R. L., GERSHWIN, L. J. & TUCKER, C. B. 2016. Fever, feeding, and grooming behavior around peak clinical signs in bovine respiratory disease. *Journal of Animal Science*, 94, 3918-3932.
- TODD, C. G., MILLMAN, S. T., LESLIE, K. E., ANDERSON, N. G., SARGEANT, J. M. & DEVRIES, T. J. 2018. Effects of milk replacer acidification and free-access feeding on early life feeding, oral, and lying behavior of dairy calves. *Journal of Dairy Science*, 101, 8236-8247.
- TOMCZAK, D. J., SAMUELSON, K. L., JENNINGS, J. S. & RICHESON, J. T. 2019. Oral hydration therapy with water and bovine respiratory disease incidence affects rumination behavior, rumen pH, and rumen temperature in high-risk, newly received beef calves. *Journal of Animal Science*, 97, 2015-2024.
- TOTH, L. A., TOLLEY, E. A. & KRUEGER, J. M. 1993. Sleep as a prognostic indicator during infectious disease in rabbits. *Proceedings of the Society for Experimental Biology and Medicine*, 203, 179-92.
- TREFZ, F. M., CONSTABLE, P. D., SAUTER-LOUIS, C., LORCH, A., KNUBBEN-SCHWEIZER, G. & LORENZ, I. 2013. Hyperkalemia in neonatal diarrheic calves depends on the degree of dehydration and the cause of the metabolic acidosis but does not require the presence of acidemia. *Journal of Dairy Science*, 96, 7234-7244.

TREFZ, F. M., LORENZ, I., LORCH, A. & CONSTABLE, P. D. 2017. Clinical signs, profound acidemia, hypoglycemia, and hypernatremia are predictive of mortality in 1,400 critically ill neonatal calves with diarrhea. *PLOS ONE*, 12, e0182938.

TSUKANO, K., LAKRITZ, J. & SUZUKI, K. 2020. Plasma histidine concentrations as a specific biomarker for intestinal mucosal damage in calves with cryptosporidiosis. *Research in Veterinary Science*, 132, 78-80.

UGWU, N., LOVE, E., MURRELL, J., WHAY, H. R., KNOWLES, T. & HOCKENHULL, J. 2021. Development of an ethogram for hutch-housed dairy calves and determination of factors influencing their behaviour. *Applied Animal Behaviour Science*, 234, 105165.

TUYTTENS, F. A. M., MOLENTO, C. F. M. & BENAÏSSA, S. 2022. Twelve Threats of Precision Livestock Farming (PLF) for Animal Welfare. *Frontiers in Veterinary Science*, Volume 9 - 2022.

UHRINCAT, M., BROUCEK, J., HANUS, A. & MACUHOVA, L. 2022. Effect of rearing, season of birth, and father on labyrinth behaviour of dairy heifers. *Journal of Animal Behaviour and Biometeorology*, 10, 1-11.

UK-VARSS (2024). Veterinary Antibiotic Resistance and Sales Surveillance Report (UK-VARSS 2023). New Haw, Addlestone: *Veterinary Medicines Directorate*

VALNÍČKOVÁ, B., STĚHULOVÁ, I., ŠÁROVÁ, R. & ŠPINKA, M. 2015. The effect of age at separation from the dam and presence of social companions on play behavior and weight gain in dairy calves. *Journal of Dairy Science*, 98, 5545-5556.

VALSALA, R. V., RANA, R., REMESH, A. T. R., THANKAPPAN, S. & BEHERA, S. 2017. Effect of *Mycoplasma bovis* on Production of Pro-Inflammatory Cytokines by Peripheral Blood Mononuclear Cells. *Advances in Animal and Veterinary Sciences*, 5, 400-404.

VAN DE GUCHT, T., SAEYS, W., VAN NUFFEL, A., PLUYM, L., PICCART, K., LAUWERS, L., VANGEYTE, J. & VAN WEYENBERG, S. 2017. Farmers' preferences for automatic lameness-detection systems in dairy cattle. *Journal of Dairy Science*, 100, 5746-5757.

VAN LEENEN, K., VAN DRIESSCHE, L., DE CREMER, L., MASMEIJER, C., BOYEN, F., DEPREZ, P. & PARDON, B. 2020. Comparison of bronchoalveolar lavage fluid bacteriology and cytology in calves classified based on combined clinical scoring and lung ultrasonography. *Preventive Veterinary Medicine*, 176, 104901.

VAN HERTEM, T., ROOIJAKKERS, L., BERCKMANS, D., PEÑA FERNÁNDEZ, A., NORTON, T., BERCKMANS, D. & VRANKEN, E. 2017. Appropriate data visualisation is key to Precision Livestock Farming acceptance. *Computers and Electronics in Agriculture*, 138, 1-10.

- VAN SELM, B., DE BOER, I. J. M., LEDGARD, S. F. & VAN MIDDELAAR, C. E. 2021. Reducing greenhouse gas emissions of New Zealand beef through better integration of dairy and beef production. *Agricultural Systems*, 186, 102936.
- VANDERMEULEN, J., BAHR, C., JOHNSTON, D., EARLEY, B., TULLO, E., FONTANA, I., GUARINO, M., EXADAKTYLOS, V. & BERCKMANS, D. 2016. Early recognition of bovine respiratory disease in calves using automated continuous monitoring of cough sounds. *Computers and Electronics in Agriculture*, 129, 15-26.
- VÁZQUEZ-DIOSDADO, J. A., DOIDGE, C., BUSHBY, E. V., OCCHIUTO, F. & KALER, J. 2024. Quantification of play behaviour in calves using automated ultra-wideband location data and its association with age, weaning and health status. *Scientific Reports*, 14, 8872.
- VELASQUEZ-MUNOZ, A., MANRIQUEZ, D., PAUDYAL, S., SOLANO, G., HAN, H., CALLAN, R., VELEZ, J. & PINEDO, P. 2019. Effect of a mechanical grooming brush on the behavior and health of recently weaned heifer calves. *BMC Veterinary Research*, 15, 284.
- VICIC, V., SALIBA, A. J., CAMPBELL, M. A., XIE, G. & QUINN, J. C. 2022. Producer practices and attitudes: Non-replacement male calf management in the Australian dairy industry. *Frontiers in Veterinary Science*, 9.
- VUPPALAPATI, C., VUPPALAPATI, R., KEDARI, S., ILAPAKURTI, A., RAMALINGAM, A., VUPPALAPATI, J. S. & KEDARI, S. 2018. Artificial Intelligence (AI) Infused Cow Necklace - For Diagnosis Of Bovine Respiratory Diseases. *2018 International Conference on Machine Learning and Cybernetics (ICMLC)*.
- WAGNER, K., BARTH, K., HILLMANN, E., PALME, R., FUTSCHIK, A. & WAIBLINGER, S. 2013. Mother rearing of dairy calves: Reactions to isolation and to confrontation with an unfamiliar conspecific in a new environment. *Applied Animal Behaviour Science*, 147, 43-54.
- WALSH, N. P., MONTAGUE, J. C., CALLOW, N. & ROWLANDS, A. V. 2004. Saliva flow rate, total protein concentration and osmolality as potential markers of whole body hydration status during progressive acute dehydration in humans. *Archives of Oral Biology*, 49, 149-154.
- WEARY, D. M., HUZZEY, J. M. & VON KEYSERLINGK, M. A. 2009. Board-invited review: Using behavior to predict and identify ill health in animals. *Journal of Animal Science*, 87, 770-7.
- WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2023. Review: Using animal welfare to frame discussion on dairy farm technology. *animal*, 17, 100836.
- WELK, A., CANTOR, M. C., NEAVE, H. W., COSTA, J. H. C., MORRISON, J. L., WINDER, C. B. & RENAUD, D. L. 2025. Effect of nonsteroidal anti-inflammatory drugs on

neonatal calf diarrhea when administered at a disease alert generated by automated milk feeders. *Journal of Dairy Science*, 108, 1842-1854.

WELK, A., OTTEN, N. D. & JENSEN, M. B. 2023. Invited review: The effect of milk feeding practices on dairy calf behavior, health, and performance: A systematic review. *Journal of Dairy Science*, 106, 5853-5879.

WENGE, J., STEINHOFEL, I., HEINRICH, C., COENEN, M. & BACHMANN, L. 2014. Water and concentrate intake, weight gain and duration of diarrhea in young suckling calves on different diets. *Livestock Science*, 159, 133-140.

WHALIN, L., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2018. Short communication: Pair housing dairy calves in modified calf hutches. *Journal of Dairy Science*, 101, 5428-5433.

WHITE, B. J., ANDERSON, D. E., RENTER, D. G., LARSON, R. L., MOSIER, D. A., KELLY, L. L., THEURER, M. E., ROBÉRT, B. D. & WALZ, M. L. 2012. Clinical, behavioral, and pulmonary changes in calves following inoculation with *Mycoplasma bovis*. *American Journal of Veterinary Research*, 73, 490-497.

WILLIAMS, M. L., MAC PARTHALAIN, N., BREWER, P., JAMES, W. P. J. & ROSE, M. T. 2016. A novel behavioral model of the pasture-based dairy cow from GPS data using data mining and machine learning techniques. *Journal of Dairy Science*, 99, 2063-2075.

WILLIAMS, P. & GREEN, L. 2007. Associations between lung lesions and grade and estimated daily live weight gain in bull beef at slaughter. *Cattle Practice*, 15, 244-249.

WILLIAMSON, H. F. & HARTLEY, S. 2024. Responsible development of digital livestock technologies for agricultural challenges: Purpose, practicality and effects are key considerations. *Sociologia Ruralis*, 64, 662-684.

WILMS, J. N., VAN DER NAT, V., GHAFARI, M. H., STEELE, M. A., SAUERWEIN, H., MARTÍN-TERESO, J. & LEAL, L. N. 2024. Fat composition of milk replacer influences growth performance, feeding behavior, and plasma fatty acid profile in ad libitum fed calves. *Journal of Dairy Science*, 107, 2797-2817.

WILMS, J. N., GHAFARI, M. H., STEELE, M. A., SAUERWEIN, H., MARTÍN-TERESO, J. & LEAL, L. N. 2022. Macronutrient profile in milk replacer or a whole milk powder modulates growth performance, feeding behavior, and blood metabolites in ad libitum-fed calves. *Journal of Dairy Science*, 105, 6670-6692.

WILSON, D. J., HABING, G., WINDER, C. B. & RENAUD, D. L. 2023. A scoping review of neonatal calf diarrhea case definitions. *Preventive Veterinary Medicine*, 211, 105818.

WINDEYER, M. C., LESLIE, K. E., GODDEN, S. M., HODGINS, D. C., LISSEMORE, K. D. & LEBLANC, S. J. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Preventive Veterinary Medicine*, 113, 231-40.

WOLF, S. A. & WOOD, S. D. 1997. Precision Farming: Environmental Legitimation, Commodification of Information, and Industrial Coordination. *Rural Sociology*, 62, 180-206.

WOODRUM SETSER, M. M., NEAVE, H. W. & COSTA, J. H. C. 2024. Are you ready for a challenge? Personality traits influence dairy calves' responses to disease, pain, and nutritional challenges. *Journal of Dairy Science*, 107, 9821-9838.

WORKMAN, A. M., KUEHN, L. A., MCDANELD, T. G., CLAWSON, M. L. & LOY, J. D. 2019. Longitudinal study of humoral immunity to bovine coronavirus, virus shedding, and treatment for bovine respiratory disease in pre-weaned beef calves. *BMC Veterinary Research*, 15, 161.

WOTTLIN, L. R., CARSTENS, G. E., KAYSER, W. C., PINCHAK, W. E., PINEDO, P. J. & RICHESON, J. T. 2021. Efficacy of statistical process control procedures to monitor deviations in physical behavior for preclinical detection of bovine respiratory disease in feedlot cattle. *Livestock Science*, 248.

YAMADA, M., ALY, S. S., DUBROVSKY, S., KARLE, B., VAN EENENNAAM, A., DONLON, J. D. & PANDIT, P. S. 2025. Effects of short-term climatic conditions on the risk of bovine respiratory disease in preweaned calves on California dairies. *Preventive Veterinary Medicine*, 245, 106658.

YAMAGUCHI, E., HAYAMA, Y., SHIMIZU, Y., MURATO, Y., SAWAI, K. & YAMAMOTO, T. 2021. Additive Bayesian network analysis of the relationship between bovine respiratory disease and management practices in dairy heifer calves at pre-weaning stage. *BMC Veterinary Research*, 17, 360.

YOUSFI, M., JANSSEN, M., DERKS, M. & VAN DER TOL, R. Innovative Off-Body Monitoring of Dairy Calves' Vital Signs for Early Disease Detection: A Thermal Imaging Approach. 11th European Conference on Precision Livestock Farming, 2024. 1038-1045.

ZEINELDIN, M., GHANEM, M., ABD EL-RAOF, Y. & ELATTAR, H. 2017. Clinical Utilization of Point-of-Care Blood L-Lactate Concentrations in Naturally Occurring Respiratory Disease in Feedlot Cattle. *Pakistan Veterinary Journal*, 37, 210-214.

ZENG, F., LI, B., WANG, H., ZHU, J., JIA, N., ZHAO, Y. & ZHAO, W. 2023. Detection of calf abnormal respiratory behavior based on frame difference and improved YOLOv5 method. *Computers and Electronics in Agriculture*, 211, 107987.

ZHANG, C., JUNIPER, D. T. & MEAGHER, R. K. 2021. Effects of physical enrichment items and social housing on calves' growth, behaviour and response to novelty. *Applied Animal Behaviour Science*, 237.

ZHANG, H., WANG, Y., CHANG, Y., LUO, H., BRITO, L. F., DONG, Y., SHI, R., WANG, Y., DONG, G. & LIU, L. 2019. Mortality-Culling Rates of Dairy Calves and Replacement Heifers and Its Risk Factors in Holstein Cattle. *Animals*, 9.

Chapter 2 Intrinsic calf factors associated with the behaviour of healthy pre-weaned group-housed dairy-bred calves.

2.0 Preface

In Chapter 1 the literature on several factors that may be associated with calf behaviour, including sex and breed were outlined. However, the literature was sparse and there was very little on variables that can be measured using the available technologies such as automatic milk feeders and accelerometers.

In the ten years between 2013 and 2023 the number of beef-sired calves born to dairy cows in GB increased by 77%, furthermore there was a commitment by the dairy industry to eliminate bull calf euthanasia between 2020 and 2023 (Ruminant Health & Welfare Group, 2025). Of the cattle slaughtered between twelve and thirty months of age in 2023, 43% were from a dairy dam, which represents an increase from 40% in 2019 (Clarke, 2024). The animals classified as dairy-beef had increased from 28% to 35% of the animals slaughtered. Thus, artificially reared beef-sired animals are of increasing importance not only to the dairy farmer but to the beef industry. A similar challenge was identified in pigs by Bus *et al.* (2021) who suggested a need to understand the effects of disease on detailed parameters such as feeding frequency considering factors such as age and breed to enable automated disease detection to be more accurate.

The existing research on the association between disease and activity and milk feeding variables only used dairy-sired animals, and most of the work was on heifers only. Thus, when designing an analysis of the association between disease and activity and milk feeding variables, it struck me that I had to make large assumptions when choosing which calf factors to pair healthy and diseased calves on. While the existing literature controls for these factors during calf recruitment all the research in this thesis is carried out under commercial conditions, on a farm that breeds a high percentage of cows to beef. Therefore, to understand the calf factors I may need to control for both in my pairing and in my models I decided to

analyse healthy calf behaviour first. The objective of this chapter was to understand the association between intrinsic calf factors and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves to inform pairing criteria and factors for inclusion in mixed models when analysing the association between these variables and disease.

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The data collection for this study was conducted as part of the WELLCALF study an INNOVATE UK funded study that focused on developing an ear tag for disease detection in calves. The calves included in this study were kept under normal farm conditions at the SRUC Dairy and Innovation Research Centre, Crichton Royal Farm, Dumfries. As part of the study daily Wisconsin calf health scoring (WCHS) was carried out by the research technicians at the farm and the calves wore an independent leg mounted sensor and used the farms milk feeding system. Data collection was supervised by Marie Haskell and David Bell and commenced in the August prior to me starting as a PhD candidate. I devised the research questions following my reading. I then planned the analysis with assistance from my co-authors. I processed and cleaned the data and performed the data analysis, produced the original draft of the paper and carried out most of the editing.

The supplementary material for this chapter can be found in Appendix 1.



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Intrinsic calf factors associated with the behavior of healthy pre-weaned group-housed dairy-bred calves

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Technology-derived behaviors are researched for disease detection in artificially-reared calves. Whilst existing studies demonstrate differences in behaviors between healthy and diseased calves, intrinsic calf factors (e.g., sex and birthweight) that may affect these behaviors have received little systematic study. This study aimed to understand the impact of a range of calf factors on milk feeding and activity variables of dairy-bred calves. Calves were group-housed from ~7 days to 39 days of age. Seven liters of milk replacer was available daily from an automatic milk feeder, which recorded feeding behaviors and live-weight. Calves were health scored daily and a tri-axial accelerometer used to record activity variables. Healthy calves were selected by excluding data collected 3 days either side of a poor health score or a treatment event. Thirty-one calves with 10 days each were analyzed. Mixed models were used to identify which of live-weight, age, sex, season of birth, age of inclusion into the group, dam parity, birthweight, and sire breed type (beef or dairy), had a significant influence on milk feeding and activity variables. Heavier calves visited the milk machine more frequently for shorter visits, drank faster and were more likely to drink their daily milk allowance than lighter calves. Older calves had a shorter mean standing bout length and were less active than younger calves. Calves born in summer had a longer daily lying time, performed more lying and standing bouts/day and had shorter mean standing bouts than those born in autumn or winter. Male calves had a longer mean lying bout length, drank more slowly and were less likely to consume their daily milk allowance than their female counterparts. Calves that were born heavier had fewer lying and standing bouts each day, a longer mean standing bout length and drank less milk per visit. Beef-sired calves had a longer mean lying bout length and drank more slowly than their dairy sired counterparts. Intrinsic calf factors influence different healthy calf behaviors in different ways. These factors must be considered in the design of research studies and the field application of behavior-based disease detection tools in artificially reared calves.

KEYWORDS

activity, behavior, calf/calves, feeding, group-housed, healthy

1. Introduction

Calves born on most dairy farms are separated from their dams and reared artificially (1). Disease is a major concern in these calves, with studies suggesting that 9–48% of artificially reared calves have bovine respiratory disease and 19–46% have diarrhea in the first 9 weeks of life (2, 3). Recently, targets have been set for reductions in both calf mortality and antibiotic use in the first 6 months of life (4). The key to a reduction in the mortality, welfare, financial and production costs of these diseases is early diagnosis. Thus, early diagnosis of disease is an area of considerable research. The use of behavior-based disease detection tools for calves has been increasingly researched (5). It is becoming more common to keep artificially-reared calves in group housing, with the provision of milk through automatic milk feeders. This provides us with the opportunity to record individual calf feeding behaviors. Similarly, the use of animal-mounted sensors is increasing globally. These technologies can aid with disease detection, for example tri-axial accelerometers can be utilized to record activity variables such as lying and standing time (6). Daily lying time and mean lying bout length have been shown to increase in calves with bovine respiratory disease (7), and a reduction in milk consumption has also been seen (8). Calves with neonatal calf diarrhea have been shown to increase lying bout length and decrease lying bout frequency in the days prior to disease development (9) in addition to drinking less milk, and drinking more slowly than their healthy counterparts (10). Recent work has utilized calf behavior to predict both neonatal calf diarrhea (11) and bovine respiratory disease and these models may include calf factors such as live-weight (12).

While there is a considerable body of literature analyzing the effects of group housing, nutrition, and disease on calf behavior in the pre-weaned period, there is very little in the published literature regarding the effects of calf factors such as age, live-weight, and breed on behavior in the pre-weaned period. Many previous studies have been designed to control factors such as age (13), sex (7, 8) and breed (6), frequently by only using one breed or sex. Thus, there is very little information to support our assumptions that these intrinsic calf factors require controlling for or as to which factors should be used in predictive models. For instance, age of the calf has been shown to influence behavior in the limited amount of literature available. Calves had more lying bouts and less time at the feeder at 40 days of age than at 26 days of age (14). Standing time has been shown to increase by 0.52 min per day of age (15). Age has also been shown to influence behavior during transport of calves <10 day old (16).

While there is literature available on the effect of breed and sex on, for example, flight speed, behavior in the handling chute (17), avoidance behaviors (18) and curiosity (19), there is no research into the effects of calf breed on lying and feeding behaviors in the pre-weaned period. One previous study has analyzed differences in activity variables between sexes with female calves spending more time walking than their male counterparts but no differences in lying or standing time (20). Another factor that affects calf behavior is the social environment. Calves that were group-housed from 3 days of age had more social interactions and performed more play behaviors than those grouped from 7 or 14 days of age (21). However, there was no information given on activity and feeding behaviors.

A variety of environmental factors may also influence calves, including heat stress, with calves in a non-shaded environment changing posture more frequently than those in the shade (22). However, to the authors' knowledge, there is no published information on whether the behavior of pre-weaned calves is affected by season in the UK climate. One study did show that calves were more likely to seek refuge at higher induced wind speeds (23), but there was no work looking at transitions between lying and standing or time spent lying or standing.

Maternal factors may also influence the calf. The pre-natal environment may influence factors such as birthweight, dystocia, immune function, and calf survival (24). Calves born to dams suffering from metabolic stress in late lactation have lower birthweights and an increased basal inflammatory response compared to calves born to dams experiencing a lower level of metabolic pressure (25). Another study found increased inflammation and metabolic stress in calves born to cows with a low body condition score 24 days prior to calving (26). Heifers born to primiparous mothers were found to be at higher risk of both mortality and culling prior to first calving than those born to multiparous mothers (27). Calf lying behavior may be influenced by maternal management during the dry period with calves born to cows kept at pasture during the dry period having longer lying periods than calves born to cows kept indoors but exercised daily (28). Calves born to dams that suffered heat stress in late lactation have altered standing variables when compared to calves born to cows that did not suffer from heat stress in late lactation (29). Furthermore, these effects may be long lasting, as heat stress in late gestation has been found to effect the milk production of both the heifer born from that gestation and the production of the heifer's daughter (30). Disease during the lactation where the heifer was conceived is associated with an increase in age at first calving and a reduction in second lactation fat yield (31).

As detailed above, the evidence suggests that several calf, dam, and environmental characteristics can affect behavior. As changes in behavior are being used in studies that aim to detect disease, we need to determine what factors are associated with key behaviors and what the size of these associations are, so that they may be accounted for in future study designs. To identify calf factors that may need to be controlled for in comparison studies or included in predictive models, an understanding of how these factors are associated with feeding and activity variables in a healthy pre-weaned calf is needed. This study aims to address this gap by analyzing the association of intrinsic calf factors with behavior in healthy pre-weaned artificially reared calves.

2. Materials and methods

2.1. Animals and housing

This work was conducted as part of the wider WELL-CALF project (UKRI project ref. 105143) under the approval of the Animal Experiment Committee of SRUC (DAI AE 10-2020) and in accordance with the Animals (Scientific Procedures) Act 1986. Calves were sourced from two sub-herds (A and B) that are kept on a single holding at SRUC Dairy Research and Innovation Center, Crichton Royal Farm, Dumfries, UK. Calves born between 07.24.20

and 01.11.21 were included in the trial. Due to a long-term trial examining genetic and environmental interactions, calves from sub-herd A came from two distinct Holstein lines: a commercial line and a high yielding line. Only male Holstein calves were included from this farm. Sub-herd B consisted of a mixture of Holstein lines. Of the 114 calves recruited into the study, forty-six had beef breed sires (Aberdeen Angus, Belgian Blue, or Longhorn) and sixty-eight had Holstein sires.

All calves received four liters of pasteurized, quality-checked thawed colostrum as soon as possible after birth. As is normal practice for this farm, calves were removed from their dam in the first 24 h of life and placed in individual straw bedded hutches. Here calves received 3 liters of reconstituted acidified milk replacer in a bucket with a teat twice daily (*Maximum + acidified, Carrs Billington Ltd.*, crude protein 24%, crude oils and fats 20%, crude ash 7.5%, calcium 0.9%, sodium 0.5%, phosphorus 0.7%, mixed to 15%). Starter pellets (compounded for the farm by *Forfarmers UK Ltd. Roughton*, dry matter 82.2%, crude protein 18%, crude oils and fats 4.5%, crude fiber 10%, crude ash 10.5%, Calcium 2%, decoquinate [*Deccox 6%, Zoetis UK Ltd. Leatherhead Surrey, 50 mg/kg*]) and water were offered in buckets *ad libitum*. On the advice of the farm's veterinary surgeon all calves born between 24.07.20 and 31.12.20 received halofuginone (*Halocur 0.5 mg/ml oral solution for calves, MSD Animal Health, Walton, Milton Keynes*, 100 µg/kg live-weight by mouth) once daily from the first to sixth day of life. For calves born after 01.01.21, this was changed to paromomycin sulfate given in milk between days zero and six of life (*Parofor, Huvepharma NV, Antwerp, Belgium*, 10 g/calf/day).

Calves were moved into groups of twelve to fourteen calves and entered the study at six to thirteen days of age (mean 8.7 days, standard deviation (SD) 2 days, median 9 days). Group pens were straw-bedded and consisted of a roofed pen (5.1 m x 5.1 m) and an igloo (3.9 m x 4.4 m, 2.2 m high) (stocking density 3.1–3.6 m²/calf). Calves were offered *ad libitum* calf starter (as above) in a trough and straw in a hay rack. Each group pen of twelve to fourteen calves had access to one teat on a single automatic calf feeding unit (*custom built for this calf unit, BioControl Norway As, Grimstad Gård, Norway*). Milk was mixed as above and calves were allowed seven liters (regardless of age) between 00:00 and 23:59. However, if they had not reached this maximum, they could carry over 1.4 liters into the next day. The maximum meal size was 1.4 liters with the day being split into five feeding periods with an additional feed becoming available at the start of each period. Water was available *ad libitum*. Both water and milk lanes included a weigh-scale, and a live-weight was recorded during each visit. A daily mean of these recorded live-weights was calculated to create the live-weight variable, as previously validated (32).

Wisconsin calf health scoring was carried out as described by McGuirk (33). Scoring was done daily by three trained technicians. The technicians were trained together in the scoring by a veterinary surgeon, and then scored a pen of ten calves together. At the end of training consistency between scorers was checked by them independently scoring a separate pen of calves. To verify the consistency between scorers across the entire dataset, scores from all scorers were analyzed on the first day present in the data set for all calves (14 days of age). The largest dataset would have allowed for any bias to be detected. However, a Kruskal-Wallis test showed no significant difference between technicians (Kruskal-Wallis

chi-squared = 2.31, df = 2, $p = 0.315$). Fecal scores were dropped from the scoring system as this measure is difficult in group-housed calves and diarrhea was not the central aim of the study. During health scoring, all calves were closed into the front of the pen for the period in which scoring took place. The farm staff treated calves independent of the Wisconsin score. If a calf had a rectal temperature of $\geq 39.5^{\circ}\text{C}$ or clinical signs of respiratory disease for 2 days or more, then the farm staff were informed.

The automatic milk feeder recorded the time of each visit where milk was consumed, the length of that visit, the volume of milk consumed at the visit and the speed at which the calf drank the milk. This was used to calculate daily values for total time at milk feeder (minutes), total milk visits (n), mean milk visit length (minutes), mean milk drinking speed (g/s), volume of milk drunk/day (ml) and mean milk per visit (ml) for each calf.

At time of entry to the group pen, a tri-axial accelerometer was placed on the right hind leg (*IceQube, IceRobotics, Edinburgh*). The IceQube accelerometer was previously validated in group housed calves that were 55 days old (34). The Motion Index (MI) is a proprietary indicator calculated by IceRobotics from the raw accelerometer data and has no units. The data output for the IceQube gave the number of transitions up (from lying to standing), the number of transitions down (from standing to lying), the time standing, the time lying and the motion index for each 15-min period. This was used to calculate daily values for the daily lying time (minutes), daily standing time (minutes), daily lying bouts (n), daily standing bouts (n), total daily motion index, mean lying bout length (minutes), mean standing bout length (minutes) and mean motion index per standing bout for each calf.

2.2. Data handling

Initial data handling was performed in Microsoft Excel with activity, feeding and health score data all being converted to this format. The day of entry to the pen was removed from all data to ensure full 24-h periods were available for analysis. Days where sick calves had been removed to individual pens for additional nursing were also removed. Only data from the day after entry to the group pen until the day prior to the commencement of weaning (39 days of age) was included for analysis. Calves were moved daily by technicians for health scoring thus 2 h of data were removed from each day's activity data. A period was chosen that accounted for 90% of the scoring times (7:15–9:15 a.m.). Data processing was carried out in R (35) using the R Studio graphical interface (*R studio, Boston, Massachusetts*) using the tidyverse package (36). Descriptive statistics were calculated in R studio using the psych package (37).

From the 114 calves on trial, seven were excluded from analysis. Reasons for exclusion were as follows; reluctant to drink ($n = 1$), mortality due to bloat ($n = 1$), congenital heart defect ($n = 1$), ear tag infection/iatrogenic ear droop ($n = 3$) and a faulty IceQube ($n = 1$). Another nine calves did not remain in the pen until weaning. Reasons for this included mortality ($n = 3$), removed to individual pen for nursing care ($n = 4$), lameness ($n = 1$) and ear swelling ($n = 1$). Eight calves had missing activity data due to equipment

problems or calves not spending sufficient time in proximity to the base station (4.8% of total calf days). 3 days of feeding data were missing due to technical problems. One calf had no birthweight recorded.

A daily total Wisconsin score for each calf was calculated using the rectal temperature, cough, and nasal discharge scores and whichever was highest of either the ear or eye scores as previously described (33). Healthy calves were identified as those with a Wisconsin score of ≤ 3 . This threshold was chosen based on that used by McGuirk and Peek (38) for healthy (≤ 3), intermediate (4) and diseased calves (≥ 5). For this study the aim was to create a balanced data set including only periods of time where a calf could be deemed as healthy without evidence of developing or recovering from disease. To do this, 3 days either side of any Wisconsin score > 3 , temperature score > 2 or veterinary treatment were removed from the dataset. Calves with at least ten consecutive healthy days were included ($n = 31$) and those with below ten consecutive health days ($n = 59$) removed. Where the healthy period was longer than ten days, the middle ten days of the healthy period were taken. The breakdown of herd, sex, and sire breed type for these thirty-one calves is shown in the (Supplementary Table 1). From these 310 calf days, seven calf days (4 calves) of activity data were missing due to technical problems. One calf had no birthweight recorded but was still included in the analysis.

2.3. Data analysis

Mixed modeling on the 310 calf days in the data set was undertaken using the lme4 package of R (39). To determine which factors to include in the multivariable model for each behavior, a univariable linear mixed effects model was constructed for each calf behavior by intrinsic factor combination (Table 1). Animal identity was nested within group as a random effect to allow for repeated measures on each calf. The factors analyzed were sex, live-weight, age, sire breed type, birthweight, season of birth, age when transferred to the group pen and dam lactation. Dam lactation was classified as 1, 2, or 3+ and sire breed type as beef or dairy. Calves born in July and August were classed as being born in summer, those born in September, October and November as born in autumn and those born in December and January as born in winter. Sub-herd was not tested, as of the eighteen calves from sub-herd A included in the study seventeen were male Holstein calves (Supplementary Table 1). *P*-values were calculated using the summary() function in the lmerTest package (40). All variables that had a $p < 0.2$ in the univariable analysis were taken forward into the multivariable analysis. A forward stepwise approach was used, and final model selection was carried out based on the Akaike information criterion corrected for small sample sizes (AICc, aictab(), AICcmodavg) (41). Where highly correlated variables were both significant at the univariable stage (i.e., age and live-weight and live-weight and birthweight see Supplementary Table 1), model building was performed with each of those variables separately, with the final model selected using AICc. Residuals were visually checked using a q-q plot produced using the DHARMA package (42) where appropriate. For those

behaviors where residuals were not normally distributed, then a log or square transformation was conducted. Mean lying bout length, mean standing bout length, total milk visits and mean milk visit length were analyzed using a natural log transformation. Mean milk drinking speed was analyzed using a square transformation. No transformation was necessary for daily lying bouts, daily standing bouts, total daily MI, mean MI per standing bout and mean milk per visit. The summary() function was used to calculate estimates and *p*-values for each model, the anova() function to calculate the numerator degrees of freedom (ndf) and denominator degrees of freedom (ddf), the confint() function was used to calculate the confidence intervals (CI) of the estimates (39) and where necessary, the exp() or sqrt() functions were used to back transform estimates and confidence intervals. The emmeans package (43) was used to calculate estimated marginal means (EMM), pairwise comparisons, *p*-values and the associated standard errors. EMM are calculated from the model, considering the other variables within the model, and give each cell in the reference grid equal weights which is advantageous in unbalanced datasets. These were then plotted using ggplot (44) to allow visualization of the results.

As these calves were fed a restricted level of milk, there was an extreme right skew to the data for the daily milk volume consumed and hence this data was not suitable for analysis using a linear mixed model. Thus, the data was converted into a binary variable of whether a calf drank its daily milk allowance or not. Calves that had drunk over 6.5 L were classed as eating their full daily allowance and coded as 1 and those eating less than this were coded as 0 for each day. This data was then analyzed using a binomial generalized linear mixed model. Model building and selection was conducted using the same method as the models above. Group and animal number were included as nested random effects. Overdispersion was checked for using the equation suggested by Bolker (45). The odds ratio and CI were then calculated using base R (35).

For behaviors where the model residuals were non-normal after transformation, multinomial mixed modeling was conducted using the generalized linear mixed modeling function in GenStat (19th Edition, VSN International, Hemel Hempstead, Hertfordshire, 2020). Model building was conducted using a forward stepwise approach using the variables that had $p < 0.2$ at the screening stage. Daily lying time, daily standing time and total time at milk feeder were analyzed in this way.

3. Results

For the thirty-one calves included in the analysis, the mean age was 27.7 days (range 11–39 days) and the mean live-weight 56.5 kg (range 41.9–79.9 kg). For further descriptive statistics of the intrinsic factors, please see, Supplementary Table 3. Descriptive statistics for each feeding and activity variable are available in Supplementary Table 3.

Univariable analysis identified thirteen out of fourteen behaviors as suitable to move forward to the model building stage (Table 1). The factors tested were sire breed type, season of birth, sex, birthweight, age at inclusion into the group pen, age, live-weight, and dam lactation number. All factors tested

TABLE 1 The effects of calf and dam characteristics on activity and feeding behaviors.

Behavior	Sire breed type	Season of birth	Sex	Birthweight	Age at inclusion	Age ^d	Live-weight ^d	Dam lactation
Daily lying bouts ^a	0.137*	0.043*	0.143*	0.048*	0.528	0.359	0.840	0.591
Daily standing bouts ^a	0.131*	0.030*	0.160*	0.045*	0.525	0.492	0.991	0.625
Mean lying bout length ^a	0.077*	0.089*	0.063*	0.073*	0.647	0.562	0.859	0.743
Mean standing bout length ^a	0.263	0.035*	0.612	0.065*	0.522	0.005*	0.016*	0.502
Total daily MI ^a	0.694	0.260	0.060*	0.326	0.120*	0.002*	0.054*	0.284
Mean MI per standing bout ^a	0.718	0.423	0.571	0.706	0.027*	0.005*	0.072*	0.483
Mean milk drinking speed ^a	0.109*	0.180*	0.181*	0.692	0.395	0.011*	0.044*	0.275
Total milk visits ^a	0.558	0.364	0.702	0.121*	0.851	0.064*	<0.001*	0.064*
Mean milk visit length ^a	0.151*	0.600	0.596	0.151*	0.940	0.012*	<0.001*	0.857
Mean milk per visit ^a	0.346	0.498	0.278	0.006*	0.294	0.270	0.004*	0.051*
Daily milk allowance consumed ^b	0.350	0.687	0.022*	0.852	0.355	0.062*	0.031*	0.692
Daily lying time ^c	0.366	0.062*	0.155*	0.355	0.370	0.777	0.319	0.484
Daily standing time ^c	0.411	0.066*	0.160*	0.363	0.391	0.229	0.310	0.483
Total time at milk feeder ^c	0.414	0.218	0.711	0.852	0.891	0.598	0.779	0.328

P-values for univariable models are shown and factors taken forward for multivariable model building are depicted with an *.

^aLinear mixed model.

^bBinomial generalized linear mixed model.

^cMultinomial generalized linear mixed model.

^dAge and live-weight for each of the individual 310 calf days.

were taken forward to the model building stage for at least two of the thirteen behaviors that underwent multivariable analysis. The multinomial analysis of total time at milk feeder yielded no variables with a $p < 0.2$ at the screening stage and therefore this analysis was not taken forward to the multivariable stage. The forward model building for daily standing time yielded only a univariable result at $p = 0.066$ for season of birth (Wald = 6.06, $F = 3.03$, $ndf = 2$, $ddf = 26$) as the multivariable model did not converge, therefore this model was disregarded.

3.1. Live-weight had a major significant association with milk feeding behaviors

Heavier calves visited the milk machine more frequently ($ndf = 1$, $ddf = 84.3$, $t = 3.819$, $p < 0.001$, Figure 1A) and for a shorter visit length ($ndf = 1$, $ddf = 200.3$, $t = -3.673$, $p < 0.001$, Figure 1B) than their lighter counterparts. Heavier calves drank faster than their lighter counterparts ($ndf = 1$, $ddf = 129.7$, $t = 2.770$, $p = 0.006$, Figure 1C) and were more likely to drink their milk allowance ($z = 2.430$, $p = 0.016$, Table 3). This is illustrated in Figure 1D, which shows that the calves that had consumed their daily milk allowance were heavier on average.

3.2. Age was associated with activity variables

Older calves had a shorter mean standing bout length ($ndf = 1$, $ddf = 224.2$, $t = -2.797$, $p = 0.06$, Figure 2A) than their younger counterparts. Calves also had a lower total daily MI ($ndf = 1$, $ddf = 145.5$, $t = -3.396$, $p < 0.001$, Figure 2C) and mean MI per standing bout ($ndf = 1$, $ddf = 114.1$, $t = -3.267$, $p = 0.001$, Figure 2B) as they got older.

3.3. Season of birth was associated with activity variables

Calves born in summer had a longer daily lying time, with calves born in autumn having the lowest daily lying time ($ndf = 2$, $ddf = 3.98$, Wald = 7.94, $p = 0.031$, Figure 3A). Both autumn- and winter-born calves had fewer lying bouts/day than summer-born calves ($ndf = 2$, $ddf = 9.7$, $t = -3.259$, $p = 0.009$, and $t = -3.045$, $p = 0.015$ respectively, Figure 3B). Calves born in both autumn and winter also had fewer standing bouts each day than those born in summer ($ndf = 2$, $ddf = 9.7$, $t = -3.504$, $p = 0.006$, and $t = -3.206$, $p = 0.012$ respectively, Supplementary Figure 1). Calves born in autumn and winter had a longer mean standing bout length than calves born in summer

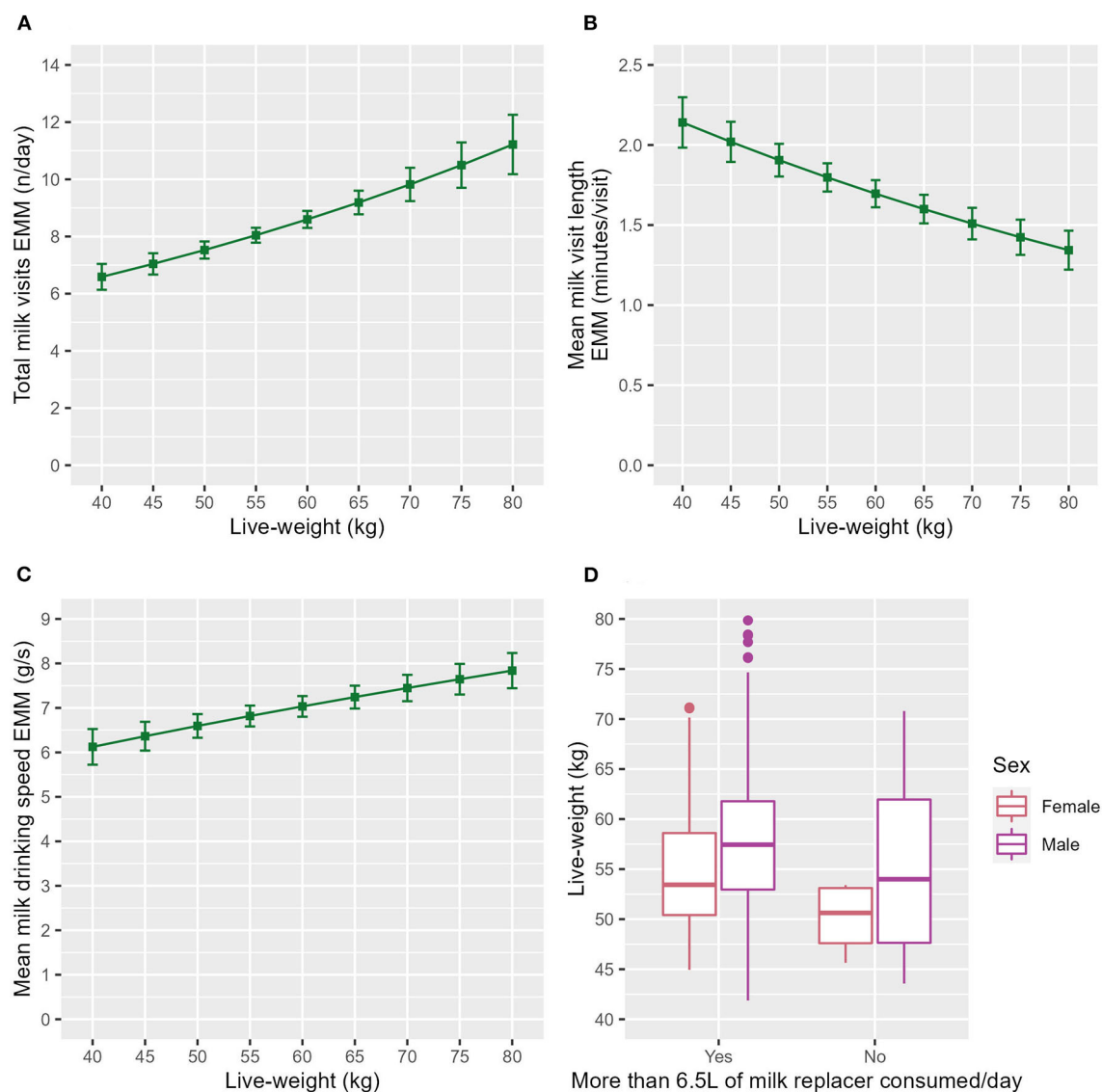


FIGURE 1
The estimated marginal means (EMM) of the (A) total milk visits, (B) mean milk visit length and (C) mean milk drinking speed in relation to live-weight. Error bars denote the standard error. (D) Box and whisker plot showing the raw data live-weight range of both sexes for the calves that had drunk their milk allowance each day and those that had not.

($ndf = 2$, $ddf = 8.4$, $t = 4.135$, $p = 0.002$, and $t = 3.263$, $p = 0.012$ respectively, [Figure 3C](#)). Season was maintained in the model for mean milk drinking speed as it improved fit but was not significant ($ndf = 2$, $ddf = 8.71$, $F = 2.264$, $p = 0.162$, [Table 2](#)).

3.4. Male calves tended to be less active and drank less

Male calves had a longer mean lying bout length ($ndf = 1$, $ddf = 22.5$, $t = 2.870$, $p = 0.009$, [Figure 4A](#)) and tended to have fewer MI units/day than female calves ($ndf = 1$, $ddf = 28.0$, $t = -1.919$, $p = 0.065$, [Figure 2B](#)). Male calves tended to have longer daily lying

time than female calves ($ndf = 1$, $ddf = 26.3$, $Wald = 7.96$, $p = 0.071$, [Table 4](#), [Figure 3A](#)).

Female calves drink faster than their male counterparts ($ndf = 1$, $ddf = 25.8$, $t = -3.033$, $p = 0.005$, [Figure 4B](#)). Male calves were much less likely to consume their milk allowance ($z = -2.530$, $p = 0.011$, [Table 3](#)) than female calves. It is important to note that in this study live-weight was not significantly associated with sex when repeat measures and group were accounted for (results not shown).

3.5. Factors with a significant but less profound association with calf behavior

Calves that were born at a higher birthweight had fewer lying ($ndf = 1$, $ddf = 25.2$, $t = -2.430$, $p = 0.023$, [Figure 3B](#))

TABLE 2 The effect of calf and dam characteristics on activity and feeding behaviors of healthy pre-weaned artificially reared calves.

Behavior	Fixed effect	Level	Effect size	Confidence interval	P-value
Daily lying bouts (<i>n</i>)	Season of birth	Summer	Reference ^b	Reference	Reference
		Autumn	-6.8	-10.6 to -3.0	0.009
		Winter	-6.5	-10.5 to -2.6	0.016
	Birthweight		-0.3	-0.5 to -0.1	0.023
Daily standing bouts (<i>n</i>)	Season of birth	Summer	Reference	Reference	Reference
		Autumn	-7.2	-11.0 to -3.4	0.006
		Winter	-6.7	-10.7 to -2.9	0.012
	Birthweight		-0.3	-0.5 to -0.1	0.021
Mean lying bout length (minutes)	Sire breed type	Beef	Reference	Reference	Reference
		Dairy	-0.8	-0.7 to -0.9	0.008
	Sex	Female	Reference	Reference	Reference
		Male	1.2	1.1-1.4	0.009
Mean standing bout length (minutes)	Season of birth	Summer	Reference	Reference	Reference
		Autumn	1.7	1.4-2.1	0.002
		Winter	1.5	1.2-1.9	0.012
	Age		-1.0	-1.0 to -1.0	0.006
	Birthweight		1.0	1.0-1.0	0.04
Total daily MI	Sex	Female	Reference	Reference	Reference
		Male	-654.0	-1,360.5-3.3	0.065
	Age at inclusion		-125.9	-276.4-34.5	0.121
	Age		-61.5	-96.2 to -25.8	<0.001
Mean MI per standing bout	Age at inclusion		-11.2	-18.9 to -2.2	0.007
	Age		-3.1	-4.9 to -1.0	0.001
Mean milk drinking speed (g/s)	Season of birth	Summer	Reference	Reference	Reference
		Autumn	2.1	-4.2-4.2	0.572
		Winter	3.8	0.7-5.3	0.112
	Sire breed type	Beef	Reference	Reference	Reference
		Dairy	4.0	2.7-5.1	0.003
	Sex	Female	Reference	Reference	Reference
Male		-3.9	-4.9 to -1.5	0.005	
Live-weight			0.8	0.4 to 1.0	0.006
Total milk visits (<i>n</i>)	Live-weight		1.0	1.0-1.0	<0.001
Mean milk visit length (minutes)	Live-weight		-1.0	-1.0 to -1.0	<0.001
Mean milk per visit (ml)	Birthweight		-13.9	-26.3 to -1.8	0.041
	Dam Lactation	1	Reference	Reference	Reference
		2	68.8	-71.7-209.6	0.363
		3+	-35.9	-185.1-110.4	0.648

The results shown are from the final linear mixed models. Factors with $p < 0.05$ are shown in bold.

^aThe results shown for the models where a transformation was used have been back transformed.

^bThe level of a categorical variable that the model uses as the "baseline."

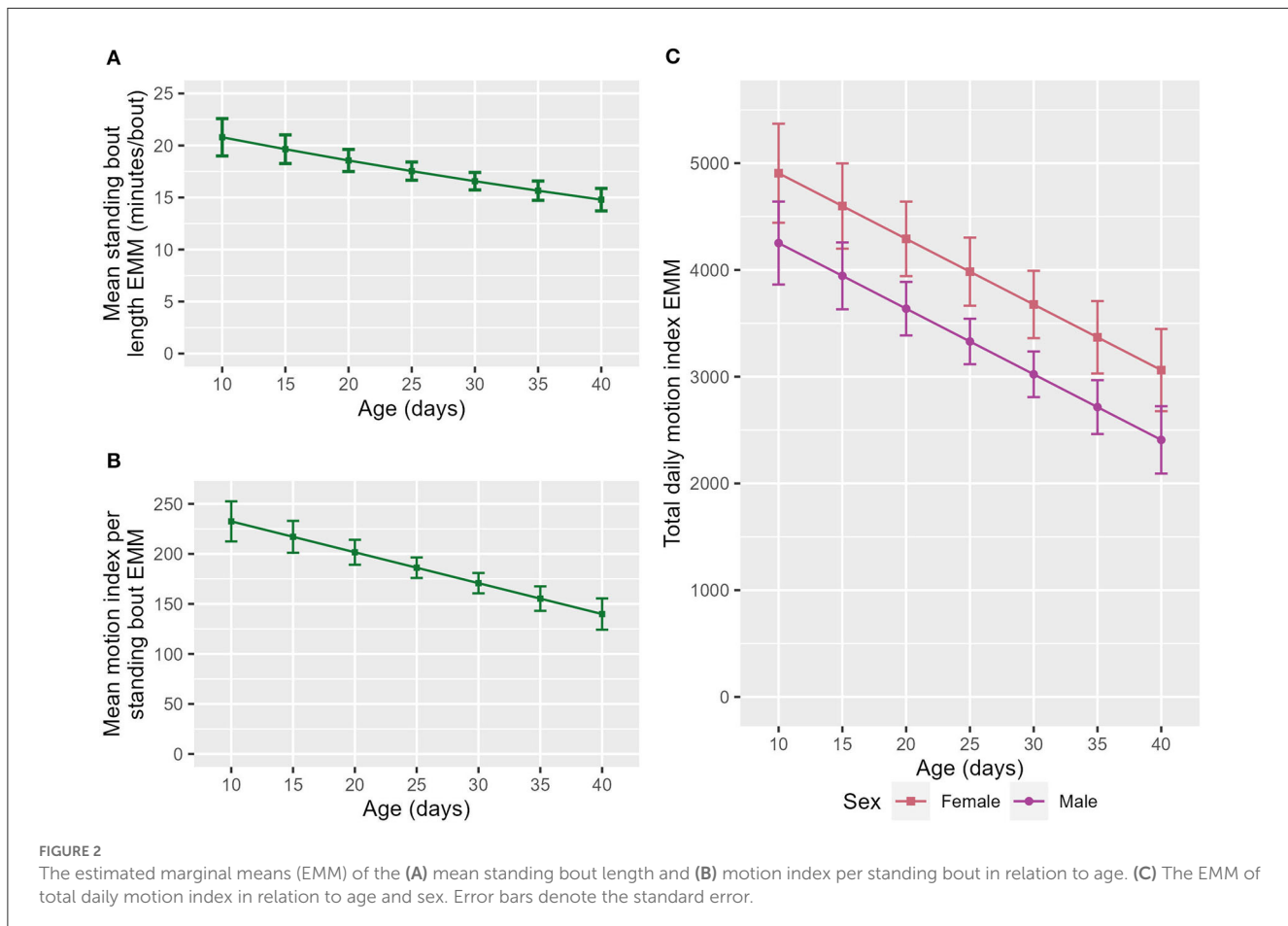
and standing ($ndf = 1$, $ddf = 25.2$, $t = -2.462$, $p = 0.006$, [Supplementary Figure 1](#)) bouts each day and a longer mean standing bout length ($ndf = 1$, $ddf = 23.9$, $t = 2.217$, $p = 0.002$,

[Figure 3C](#)). Calves that were born at a higher birthweight also drank less milk per feed ($ndf = 1$, $ddf = 24.6$, $t = -2.154$, $p = 0.041$, [Figure 5A](#)).

TABLE 3 The effect of calf characteristics on the odds ratios of the total daily milk allowance having been drunk by healthy pre-weaned artificially reared calves.

Fixed effect	Level	Odds ratio	95% Confidence interval	P-value
Sex	Female	Reference	Reference	Reference
	Male	0.08	0.01 - 0.56	0.011
Live-weight		1.13	1.02 - 1.25	0.016

Results shown are from the final binomial generalized linear mixed model output. Factors with $p < 0.05$ are shown in bold.



Compared to dairy-sired calves, calves with a beef sire had a longer mean lying bout length (ndf = 1, ddf = 21.9, $t = -2.919$, $p = 0.008$, Figure 4A). Beef-sired calves also drank more slowly (ndf = 1, ddf = 23.3, $t = 3.359$, $p = 0.003$, Figure 4B) than their dairy-sired counterparts.

Calves that were introduced into the group pen at an older age had a reduced MI per standing bout (ndf = 1, ddf = 28.0, $t = -2.949$, $p = 0.007$, Figure 5B). Age at inclusion into the group pen was also included in the final model for total MI but was not significant (ndf = 1, ddf = 26.9, $t = -1.598$, $p = 0.121$, Table 2).

Dam lactation was retained in the final model for mean milk per visit as it improved fit (ndf = 2, ddf = 24.2, $F = 1.233$, $p = 0.309$, Table 2) but was not significant in any other of the final models.

4. Discussion

This study found that live-weight, age, sex, and season of birth influenced activity and feeding behavior variables in pre-weaned artificially weaned calves. Birthweight, sire breed type and age at inclusion into the group pen did have significant associations but with fewer behaviors. Dam lactation had no association with behavior in pre-weaned calves.

The association of live-weight with healthy calf behavior has not previously been explored. However, some studies have used live-weight to pair diseased and healthy calves e.g., Duthie et al. (7) and included in disease prediction models e.g., Chisholm et al. (46). The current study found that heavier calves visited the milk machine more frequently (Figure 1A), for a shorter visit length (Figure 1B), drank faster (Figure 1C) and were slightly more likely to drink

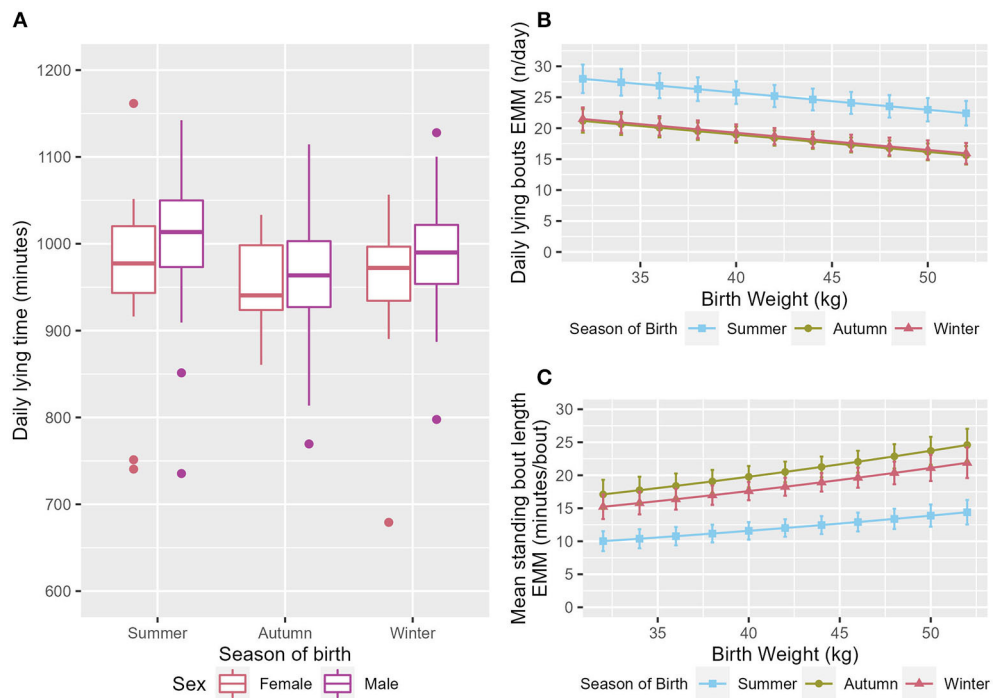


FIGURE 3 (A) Box and whisker plot of the raw data for daily lying time showing the range of values across the three seasons and both sexes. The EMM of the (B) daily lying bouts, (C) mean standing bout length in relation to season and birthweight. Error bars denote the standard error.

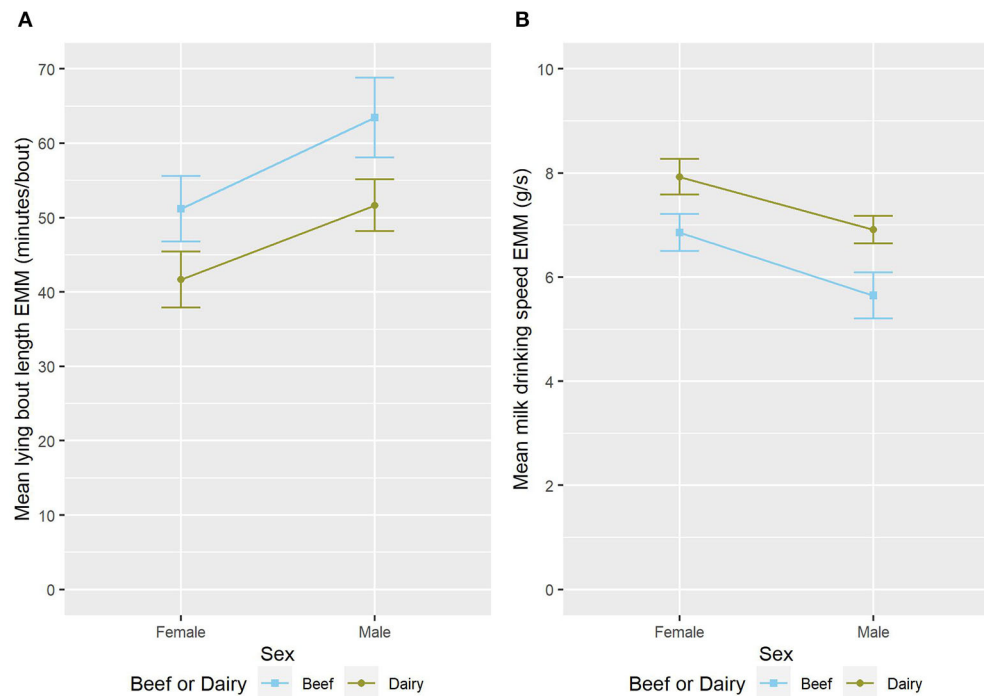


FIGURE 4 The estimated marginal means (EMM) of the (A) mean lying bout length and (B) mean milk drinking speed in relation to sex and sire breed type. Error bars denote the standard error.

their milk allowance (Table 3) than their lighter counterparts. This concurs with a recent study that suggested a fast-drinking

personality type is associated with increased live-weight gain (47). Another possible explanation is that due to the feed allowance being

TABLE 4 The effect of calf characteristics on the daily lying time in healthy pre-weaned artificially reared calves.

Fixed effect	Wald statistic	Numerator degrees of freedom	F statistic	Denominator degrees of freedom	P value
Season of birth	7.96	2	3.98	27.1	0.031
Sex	3.54	1	3.54	26.3	0.071

Result shown are from the final multinomial generalized linear mixed model. Factors with $p < 0.05$ are shown in bold.

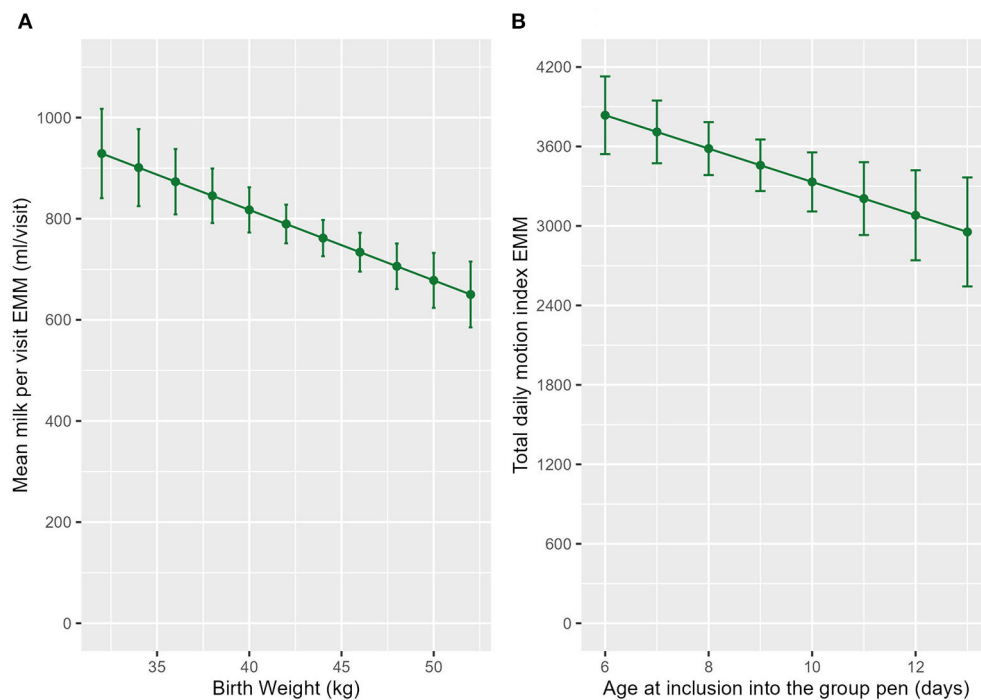


FIGURE 5

The estimated marginal means (EMM) of the (A) mean milk per visit in relation to birthweight. (B) EMM of the total daily motion index in relation to age at inclusion into the group pen. Error bars denote the standard error.

the same for all calves the larger calves were receiving a lower % of their bodyweight in milk and this was influencing their behavior. A previous study found time drinking was reduced in calves on a lower level of milk replacer (48). Calves fed less milk have been shown to visit the milk feeder more often (49).

Unlike in previous work, no increase in either the number of lying bouts (14) or daily standing time (15) with age was seen in this study. This study did, however, find that older calves had a lower total daily MI (Figure 2C), mean MI per standing bout (Figure 2B) and standing bout length (Figure 2A). This may reflect calves playing less as they get older. Calves have previously been found to have a reduction in play between 3 and 6 weeks of age (50). Another possible explanation is that stabilization of the group composition means that less movement is required. It may also reflect reduced exploration as calves become more familiar with their environment, for example, weaned calves visit different areas of the pasture more often when first introduced into a pasture but this behavior reduces over time (51).

Only one previous study has examined the association of birthweight with milk drinking behaviors and activity variables. It found that calves born at a normal (42.7 ± 2.6 kg) birthweight

spent more time drinking milk and had a greater daily standing time than their lighter (34.9 ± 2.4 kg) counterparts (52). In contrast the current study found that calves that were born heavier had fewer lying (Figure 3B) and standing (Supplementary Figure 1) bouts each day, a longer mean standing bout length (Figure 3C) and drank less milk per feed (Figure 5B). However, in the current study the range of birthweights was greater (32–52 kg). The reduction in the number of lying and standing bouts was an interesting and unexpected finding, especially as birthweight is positively associated with play behavior in piglets (53). All the calves included in this analysis had an unassisted birth, so this association is unlikely to be due to dystocia. It is worth noting that birthweight in this study was influenced by both sex ($p = 0.050$, Supplementary Table 5) and dam lactation (first lactation-second lactation $p = 0.013$, first lactation-third lactation and over $p = 0.002$, Supplementary Table 5). In turn, birthweight had a significant effect on live-weight in a mixed model ($p < 0.001$, Supplementary Table 5). Interestingly a recent study found that as well as starting at a smaller size, calves with a smaller birthweight also grow more slowly (52). A larger study size may allow examination of interactions between the factors

examined in this study, but this was not possible with only thirty-one calves.

Picking apart the associations between age, live-weight and birthweight and behavior is challenging. Live-weight is highly correlated with both age and birthweight (Supplementary Table 2). Interestingly, in Table 1 we can see that at the univariable level behaviors were only significantly ($p < 0.05$) associated with one or two of these three factors, this was unexpected due to the high level of correlation. The pattern seen in this study where when both age and weight had a $p < 0.1$ at the univariable stage, age produced a better fitting multivariable model for activity variables and weight produced a better fitting multivariable model for milk drinking behaviors was interesting. It is not however possible to pick out which of age and live-weight is more important.

Other studies have also recognized the importance of live-weight and age. For instance, both variables were previously included in random forest models for predicting bovine respiratory disease (54). It is worth noting that currently the inclusion of live-weight in predictive models for disease will require the automatic milk feeders to have a weighing platform, this is likely to require additional investment. Live-weight data also required significant data cleaning due to calves only partially standing on the scale or a second calf standing behind them. Thus, to use live-weight from a weighing platform in automatic disease detection tools automated cleaning of raw live-weight data prior to the disease detection model would be required or feeder design would need to be altered to ensure only a single whole calf could enter at any one time. Existing auto-weighers on the market already incorporate this data processing. An alternative would be to use a computer vision based system such as that used by Cominotte et al. (55) to estimate body weight. Using birthweight may be simpler and require less investment than live-weight. Thus, birthweight warrants further investigation as a possible alternative to live-weight for use in disease detection tools, possibly in conjunction with age.

In this study calves born in summer had a longer daily lying time (Figure 3A), more lying and standing bouts/day (Figure 3B and Supplementary Figure 1), and a shorter mean standing bout length (Figure 3C) than calves born in autumn or winter. It is highly likely that these associations are environmental in nature. The changes seen in the number of lying and standing bouts is consistent with the previous findings of differences in the number of posture changes in different levels of shade (22). The same study found no effect of shade on lying time however which does not concur with the finding that summer born calves had a longer daily lying time (Table 4) in the current study. The temperature and humidity index and the use of a ventilation system has been shown to influence the behaviors of 15 month old bulls (56), although the current study was conducted at far lower ambient temperatures (-5.5 – 28.2°C in current study compared to 20 – 30°C in bull study). Calves have been previously found to be aversive to wind (23) which may be reflected in the change in behavior in the autumn and winter. However, the association seen here is the opposite to what the authors would have expected. The associations with season in this study may not be completely due the conditions experienced by the calf. Calves born to cows that have been subject to heat stress in late lactation have been shown to have shorter standing bouts in the first week of life and shorter more frequent standing bouts

in weeks seven and eight of life when compared to calves born to cows that had been cooled in late lactation (29). This is consistent with the findings of this experiment. This study has identified that season/environmental factors should be considered as it may alter behaviors. However, this analysis is limited by the fact that no calves included in this study were born between February and July and this should be considered for further studies. It may also be that future disease detection tools will also need to take into account factors such as substrate, which has been previously shown to affect locomotor play in an arena test (57).

The finding of a tendency for increased activity (Total Daily MI) in female calves in this study concurs with a previous finding that female 6-week-old calves spend more time walking than their male counterparts (20) and may reflect the increased reaction to human approach previously seen in female calves (18). Interestingly, previous work has shown that male calves required fewer training sessions to use an automatic milk feeder than female calves (58). This contrasts with the finding in this study that male calves tended to drink slower (Figure 4B) and were much less likely to consume their daily milk allowance (Table 3). However due to the small sample size the interaction between sex and age was not tested so while this may be an effect of learning to use the feeder this cannot be confirmed.

Previous studies investigating effects of calf breed have not used either the same breeds or the same behaviors as the current study. The association of sire breed type with mean lying bout length (Figure 4A) and drinking speed (Figure 4B) seen in this study may reflect an effect of the calf's personality on their behavior. Previous authors have studied breed differences in some personality traits. For example, a study has previously shown that Holstein calves had a more fearful response in the escape test when compared to Holstein beef cross calves (17). Previous work using Holstein calves looked at three personality types, interactive, exploratory-active, and vocal inactive and found that more vocal calves have a greater number of rewarded visits to the feeder pre-weaning (59). It was not possible in this study to explore the interaction between breed and sex. This is due both to the small sample size and the skewed distribution between sex and breed. This occurred in part as an artifact of the data selection process and in part due to the breeding policies of the two sub-herds.

The association of age at inclusion into the group pen with mean MI per standing bout (Figure 5C) may reflect the reduction in social interaction seen in calves grouped at an older age (21). It is unlikely however that the change in behavior purely reflected the initial post grouping period however as calves were grouped at a maximum of 13 days of age (Mean 8.7 days, Supplementary Table 3) while the mean age of the data included in the final data set was 27.7 days. It was an interesting finding that age at inclusion into the group pen was not significant for any milk feeding behaviors in this study. Calves introduced to the automatic milk feeder at less than a day of age have previously been found to drink less milk in the first week of life than those fed three times daily by bottle until 5 days of age, however this effect was not seen after 8 days of age (58). This may explain why no association with milk feeding behaviors were seen in this study, as the days immediately after introduction to the group pen were not included. However, another study found that the frequency of rewarded visits

was significantly lower in the first 12 days in the group pen in calves that were introduced at 6 days of age compared to those introduced at fourteen days of age, thus there may be an effect of age at introduction for a longer time period after introduction (60). Interestingly, in a study with a slightly lower age at inclusion into the group pen (mean 7.8 days, SD 1.9 days compared to mean 8.7 days, SD 2 days in this study) a higher age at inclusion was associated with an increase in the age at first calving (61).

Dam lactation was tested in this study as a proxy for maternal management as heifers will be managed differently to other cows. As the calves are removed as soon as possible after birth and are not fed colostrum from their own mother, differences in maternal behavior between lactations are unlikely to influence the calf. Lactation number was not associated with any of the behaviors in this study (Tables 2–4). This may be due to an insufficient difference in management between the lactation groups, especially in late pregnancy.

Several studies have examined changes in the number of unrewarded visits to the automatic milk feeder with disease. The number of unrewarded visits has been shown to decline in the 2 days prior to neonatal calf diarrhea diagnosis (62) and the 3 days prior to bovine respiratory disease diagnosis (63). Unfortunately, the automatic milk feeder used in this study did not record unrewarded visits to the automatic feeder. A potential area of further research is to repeat this experiment with an automatic milk feeder that records both rewarded and unrewarded visits to the feeder.

A further limiting factor of this study is the size of dataset. Thirty-one calves out of a possible 114 calves were included with ten calf days each. Gathering a sufficient data set of healthy calves is challenging due to the high incidence of disease in young calves. Across eleven UK dairy farms, on farm incidence of bovine respiratory disease and neonatal calf diarrhea in the first 9 weeks of life were 20.4–77.8% and 24.1–74.4%, respectively (2). While more relaxed inclusion criteria could have been used, the authors deemed that it was vital to remove any confounding caused by disease due to its strong effect on behavior. The small dataset is likely to particularly impact the investigation of the effect of lactation as studies into maternal factors would frequently analyze far larger datasets, e.g., 60 calves (29), 551 calves (64) or 15,992 calves (31). However, some studies have used a smaller number of calves, Ling et al. (25) used twelve calves in their study. Larger datasets are likely to be beneficial especially when attempting to identify subtle changes in behavior.

Interestingly, a recent study has shown that individual repeatability differs between milk feeding behaviors even after adjustment for age, cohort and day number (65). This could mean that further studies should focus on those behaviors with a high level of intra-individual repeatability. Intriguingly, feeding rate and the number of meals were highly correlated in a previous study (47) but were not significantly correlated in this study when correlations of the entire dataset were calculated (Supplementary Table 6), yet the approach of calculating correlations for individual calves and then an overall correlation may warrant further investigation.

In recent years the use of sensors and automated systems to detect disease has been increasingly researched (5). However, the performance of the reported models for bovine respiratory disease

in pre-weaned calves is frequently poor, accuracies reported include 59.1% (66), 75% (54) and 80% (11). While no data is currently available on calf diseases, we know from previous work done on automatic tools for lameness detection in Canadian dairy herds that accuracy is a key concern for farmers (67). Farmers required a system that both detected a high proportion of lame cows and did not give many false alerts. Thus, it is vital that we have a thorough understanding of factors other than disease that are associated with changes in sensor measured behaviors in pre-weaned calves. This study aimed to address this gap in the literature and identified several factors that are associated with sensor measured behavioral variables. However, this study was conducted in a single shed; thus, it has its own environmental and management variables. To verify the results of this study the work needs to be repeated on several units.

5. Conclusion

This study found several intrinsic calf factors that are associated with healthy calf behavior. Most notable of these were live-weight, season, sex, and age. These are important considerations for comparing behavior between sick and healthy calves and predicting disease. However, there are likely to be farm level factors that also apply, and this needs further research as all calves in this study were reared in the same shed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Animal Experiment Committee of the Scottish Rural College.

Author contributions

MH, C-AD, CM, JB, and DB designed the data collection of this work. BR developed the idea for the analysis, cleaned the data, performed the analysis, and wrote the first draft of the paper. MH, C-AD, AC, and CM provided support with the data cleaning and analysis. All authors assisted with drafting of the manuscript and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Wagner K, Barth K, Hillmann E, Palme R, Futschik A, Waiblinger S. Mother rearing of dairy calves: reactions to isolation and to confrontation with an unfamiliar conspecific in a new environment. *Appl Anim Behav Sci.* (2013) 147:43–54. doi: 10.1016/j.applanim.2013.04.010
- Johnson KF, Chancellor N, Burn CC, Wathes DC. Prospective cohort study to assess rates of contagious disease in pre-weaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. *Vet Rec Open.* (2017) 4:e000226. doi: 10.1136/vetreco-2017-000226
- Dachrodt L, Arndt H, Bartel A, Kellermann LM, Tautenhahn A, Volkmann M, et al. Prevalence of disorders in preweaned dairy calves from 731 dairies in Germany: a cross-sectional study. *J Dairy Sci.* (2021) 104:9037–51. doi: 10.3168/jds.2021-20283
- RUMA. Targets Task Force 2020. (2020). Available online at: <https://www.ruma.org.uk/wp-content/uploads/2020/11/SO-469-RUMA-REPORT-021220.pdf>. RUMA (accessed February 25, 2022).
- Costa JHC, Cantor MC, Neave HW. Symposium review: precision technologies for dairy calves and management applications. *J Dairy Sci.* (2021) 104:1203–19. doi: 10.3168/jds.2019-17885
- Sun D, Webb L, van der Tol PJJ, van Reenen K, A. Systematic review of automatic health monitoring in calves: glimpsing the future from current practice. *Front Vet Sci.* (2021) 8:761468. doi: 10.3389/fvets.2021.761468
- Duthie CA, Bowen JM, Bell DJ, Miller GA, Mason C, Haskell MJ. Feeding behaviour and activity as early indicators of disease in pre-weaned dairy calves. *Animal.* (2021) 15:100150. doi: 10.1016/j.animal.2020.100150
- Swartz TH, Findlay AN, Petersson-Wolfe CS. Short communication: automated detection of behavioral changes from respiratory disease in pre-weaned calves. *J Dairy Sci.* (2017) 100:9273–8. doi: 10.3168/jds.2016-12280
- Lowe GL, Sutherland MA, Waas JR, Schaefer AL, Cox NR, Stewart M. Physiological and behavioral responses as indicators for early disease detection in dairy calves. *J Dairy Sci.* (2019) 102:5389–402. doi: 10.3168/jds.2018-15701
- Knauer WA, Godden SM, Dietrich A, James RE. The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *J Dairy Sci.* (2017) 100:5642–52. doi: 10.3168/jds.2016-12372
- Ghaffari MH, Monneret A, Hammon HM, Post C, Müller U, Frieten D, et al. Deep convolutional neural networks for the detection of diarrhea and respiratory disease in preweaning dairy calves using data from automated milk feeders. *J Dairy Sci.* (2022) 105:9882–95. doi: 10.3168/jds.2021-21547
- Cantor MC, Casella E, Silvestri S, Renaud DL, Costa JHC. Using machine learning and behavioral patterns observed by automated feeders and accelerometers for the early indication of clinical bovine respiratory disease status in preweaned dairy calves. *Front Animal Sci.* (2022) 3:2359. doi: 10.3389/fanim.2022.852359
- Belaid MA, Rodríguez-Prado M, Rodríguez-Prado DV, Chevaux E, Calsamiglia S. Using behavior as an early predictor of sickness in veal calves. *J Dairy Sci.* (2020) 103:1874–83. doi: 10.3168/jds.2019-16887
- Jensen MB, Jensen A, Vestergaard M. The effect of milk feeding strategy and restriction of meal patterning on behavior, solid feed intake, and growth performance of male dairy calves fed via computer-controlled milk feeders. *J Dairy Sci.* (2020) 103:8494–506. doi: 10.3168/jds.2020-18166

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2023.1204580/full#supplementary-material>

- Hill TM, Bateman HG II, Aldrich JM, Quigley JD, Schlotterbeck RL. Short communication: intensive measurements of standing time of dairy calves housed in individual pens within a naturally ventilated, unheated nursery over different periods of the year. *J Dairy Sci.* (2013) 96:1811–4. doi: 10.3168/jds.2012-6206
- Jongman EC, Butler KL. The effect of age, stocking density and flooring during transport on welfare of young dairy calves in Australia. *Animals.* (2014) 4:184–99. doi: 10.3390/ani4020184
- Hoppe S, Brandt HR, König S, Erhardt G, Gauly M. Temperament traits of beef calves measured under field conditions and their relationships to performance. *J Anim Sci.* (2010) 88:1982–9. doi: 10.2527/jas.2008-1557
- Ellingsen-Dalskau K, Dean KR, Rousing T. On-farm testing of dairy calves' avoidance response to human approach: effects of sex, age and test order. *Animal Welfare.* (2020) 29:411–8. doi: 10.7120/09627286.29.4.411
- Lauber MC, Hemsworth PH, Barnett JL. The effect of breed, time spent with dam and late pregnancy induction of parturition on behavioural development in dairy calves. *Behav Processes.* (2009) 82:301–11. doi: 10.1016/j.beproc.2009.07.010
- Ugwu N, Love E, Murrell J, Whay HR, Knowles T, Hockenfull J. Development of an ethogram for hutch-housed dairy calves and determination of factors influencing their behaviour. *Appl Anim Behav Sci.* (2021) 234:105165. doi: 10.1016/j.applanim.2020.105165
- Abdelfattah EM, Karousa MM, Lay DC Jr, Marchant-Forde JN, Eicher SD. Short communication: effect of age at group housing on behavior, cortisol, health, and leukocyte differential counts of neonatal bull dairy calves. *J Dairy Sci.* (2018) 101:596–602. doi: 10.3168/jds.2017-12632
- Kovacs L, Kezer FL, Bakony M, Jurkovich V, Szenci O. Lying down frequency as a discomfort index in heat stressed Holstein bull calves. *Sci Rep.* (2018) 8:15065. doi: 10.1038/s41598-018-33451-6
- Bell DJ, Haskell MJ, Macrae AI, Mitchell MA, Jennings A, Mason CS. Behavioural responses of dairy calves to temperature and wind. In: *Proceedings of the 53rd Annual Congress of the ISAE.* (2019).
- Perry VEA, Copping KJ, Miguel-Pacheco G, Hernandez-Medrano J. The effects of developmental programming upon neonatal mortality. *Vet Clin North Am Food Anim Pract.* (2019) 35:289–302. doi: 10.1016/j.cvfa.2019.02.002
- Ling T, Hernandez-Jover M, Sordillo LM, Abuelo A. Maternal late-gestation metabolic stress is associated with changes in immune and metabolic responses of dairy calves. *J Dairy Sci.* (2018) 101:6568–80. doi: 10.3168/jds.2017-14038
- Swartz TH, Bradford BJ, Lemke M, Mamedova LK, Agnew R, Fehn J, et al. Effects of prenatal dietary rumen-protected choline supplementation during late gestation on calf growth, metabolism, and vaccine response. *J Dairy Sci.* (2022) 105:9639–51. doi: 10.3168/jds.2022-22239
- Carvalho MR, Aboujaoude C, Peñaricano F, Santos JEP, DeVries TJ, McBride BW, et al. Associations between maternal characteristics and health, survival, and performance of dairy heifers from birth through first lactation. *J Dairy Sci.* (2020) 103:823–39. doi: 10.3168/jds.2019-17083
- Black RA, Whitlock BK, Krawczel PD. Effect of maternal exercise on calf dry matter intake, weight gain, behavior, and cortisol concentrations at disabbling and weaning. *J Dairy Sci.* (2017) 100:7390–400. doi: 10.3168/jds.2016-12191

29. Laporta J, Fabris TF, Skibieli AL, Powell JL, Hayen MJ, Horvath K, et al. In utero exposure to heat stress during late gestation has prolonged effects on the activity patterns and growth of dairy calves. *J Dairy Sci.* (2017) 100:2976–84. doi: 10.3168/jds.2016-11993
30. Laporta J, Ferreira FC, Ouellet V, Dado-Senn B, Almeida AK, De Vries A, et al. Late-gestation heat stress impairs daughter and granddaughter lifetime performance. *J Dairy Sci.* (2020) 103:7555–68. doi: 10.3168/jds.2020-18154
31. Swartz TH, Bradford BJ, Clay JS. Intergenerational cycle of disease: maternal mastitis is associated with poorer daughter performance in dairy cattle. *J Dairy Sci.* (2021) 104:4537–48. doi: 10.3168/jds.2020-19249
32. Cantor MC, Pertuisel CH, Costa JHC. Technical note: estimating body weight of dairy calves with a partial-weight scale attached to an automated milk feeder. *J Dairy Sci.* (2020) 103:1914–9. doi: 10.3168/jds.2019-16918
33. McQuirk SM. *Calf Health Scoring Chart* In: calf_health_scoring_chart, editor. (2015). School of Veterinary Medicine, Wisconsin-Madison University. Available online at: https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf (accessed February 1, 2023).
34. Finney G, Gordon A, Scoley G, Morrison SJ. Validating the icerobotics icecube tri-axial accelerometer for measuring daily lying duration in dairy calves. *Livest Sci.* (2018) 214:83–7. doi: 10.1016/j.livsci.2018.05.014
35. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing (2021).
36. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the tidyverse. *J Open Source Software.* (2019) 4:1686. doi: 10.21105/joss.01686
37. Revelle W. *Psych: Procedures for Psychological, Psychometric, and Personality Research*. Evanston, IL: Northwestern University (2021).
38. McQuirk SM, Peek SF. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. *Anim Health Res Rev.* (2014) 15:145–7. doi: 10.1017/S1466252314000267
39. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw.* (2015) 67:1–48. doi: 10.18637/jss.v067.i01
40. Kuznetsova A, Brockhoff PB, Christensen RHB. LmerTest package: tests in linear mixed effects models. *J Stat Softw.* (2017) 82:1–26. doi: 10.18637/jss.v082.i13
41. Mazerolle MJ. *AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c)*. R package version 2.3.1. (2022). Available online at: <https://cran.r-project.org/package=AICcmodavg>
42. Hartig F. *DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models*. R package version 0.4.5. (2021). Available online at: <https://CRAN.R-project.org/package=DHARMA>
43. Lenth RV. *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version 1.8.7. (2023). Available online at: <https://CRAN.R-project.org/package=emmeans>
44. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag (2016).
45. Bolker B. *Glmm Faq* <https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#overdispersion>:BenBolker. (2021). Available online at: <https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#overdispersion> (accessed December 21, 2021).
46. Chisholm E, Scoley G, Lamrock F. Using a multi-state modelling (Msm) approach to predict dairy calf health status using on-farm precision technology. *Data Animal - science Proceed.* (2021) 12:85. doi: 10.1016/j.anscip.2021.03.107
47. Carslake C, Occhiuto F, Vázquez-Diosdado JA, Kaler J. Indication of a personality trait in dairy calves and its link to weight gain through automatically collected feeding behaviours. *Sci Reports.* (2022) 12:76. doi: 10.1038/s41598-022-24076-x
48. Shiasi Saradoabi R, Alikhani M, Hashemzadeh F, Khorvash M, Mirzaei M, Drackley JK. Effects of different planes of milk feeding and milk total solids concentration on growth, ruminal fermentation, health, and behavior of late weaned dairy calves during summer. *J Anim Sci Biotechnol.* (2021) 12:96. doi: 10.1186/s40104-021-00615-w
49. Rosenberger K, Costa JHC, Neave HW, von Keyserlingk MAG, Weary DM. The effect of milk allowance on behavior and weight gains in dairy calves. *J Dairy Sci.* (2017) 100:504–12. doi: 10.3168/jds.2016-11195
50. Duve LR, Weary DM, Halekoh U, Jensen MB. The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. *J Dairy Sci.* (2012) 95:6571–81. doi: 10.3168/jds.2011-5170
51. Horvath KC, Miller-Cushon EK. Characterizing social behavior, activity, and associations between cognition and behavior upon social grouping of weaned dairy calves. *J Dairy Sci.* (2018) 101:7287–96. doi: 10.3168/jds.2018-14545
52. Mousavi-Haghshenas MA, Hashemzadeh F, Ghorbani GR, Ghasemi E, Rafiee H, Ghaffari MH. Trace minerals source in calf starters interacts with birth weights to affect growth performance. *Sci Rep.* (2022) 12:4. doi: 10.1038/s41598-022-23459-4
53. Brown SM, Klaffenböck M, Nevison IM, Lawrence AB. Evidence for litter differences in play behaviour in pre-weaned pigs. *Appl Anim Behav Sci.* (2015) 172:17–25. doi: 10.1016/j.applanim.2015.09.007
54. Bowen JM, Haskell MJ, Miller GA, Mason CS, Bell DJ, Duthie CA. Early prediction of respiratory disease in preweaning dairy calves using feeding and activity behaviors. *J Dairy Sci.* (2021) 104:12009–18. doi: 10.3168/jds.2021-20373
55. Cominotte A, Fernandes AFA, Dorea JRR, Rosa GJM, Ladeira MM, van Cleef EHC, et al. Automated computer vision system to predict body weight and average daily gain in beef cattle during growing and finishing phases. *Livest Sci.* (2020) 232:103904. doi: 10.1016/j.livsci.2019.103904
56. Parrini S, Sirtori F, Fabbri MC, Dal Prà A, Crovetto A, Bozzi R. Effects of a ceiling fan ventilation system and thi on young limousin bulls' social behaviour. *Animals.* (2022) 12:1259. doi: 10.3390/ani12101259
57. Sutherland MA, Worth GM, Schütz KE, Stewart M. Rearing substrate and space allowance influences locomotor play behaviour of dairy calves in an arena test. *Appl Anim Behav Sci.* (2014) 154:8–14. doi: 10.1016/j.applanim.2014.02.008
58. Wilson TR, LeBlanc SJ, DeVries TJ, Haley DB. Effect of stall design on dairy calf transition to voluntary feeding on an automatic milk feeder after introduction to group housing. *J Dairy Sci.* (2018) 101:5307–16. doi: 10.3168/jds.2017-14011
59. Neave HW, Costa JHC, Weary DM, von Keyserlingk MAG. Personality is associated with feeding behavior and performance in dairy calves. *J Dairy Sci.* (2018) 101:7437–49. doi: 10.3168/jds.2017-14248
60. Jensen MB. Age at introduction to the group affects dairy calves' use of a computer-controlled milk feeder. *Appl Anim Behav Sci.* (2007) 107:22–31. doi: 10.1016/j.applanim.2006.09.017
61. Swartz TH, Peterson-Wolfe CS. Associations between preweaning calf feeding behaviors with age at first calving and lactational performance using an automatic calf feeder. *JDS Commun.* (2023) 4:75–9. doi: 10.3168/jds.2022-0255
62. Sutherland MA, Lowe GL, Huddart FJ, Waas JR, Stewart M. Measurement of dairy calf behavior prior to onset of clinical disease and in response to disbudding using automated calf feeders and accelerometers. *J Dairy Sci.* (2018) 101:8208–16. doi: 10.3168/jds.2017-14207
63. Johnston D, Kenny DA, McGee M, Waters SM, Kelly AK, Earley B. Electronic feeding behavioural data as indicators of health status in dairy calves. *Irish J Agricult Food Res.* (2016) 55:159–68. doi: 10.1515/ijaf-2016-0016
64. Immler M, Büttner K, Gärtner T, Wehrend A, Donat K. Maternal impact on serum immunoglobulin and total protein concentration in dairy calves. *Animals.* (2022) 12:755. doi: 10.3390/ani12060755
65. Carslake C, Occhiuto F, Vázquez-Diosdado JA, Kaler J. Repeatability and predictability of calf feeding behaviors-quantifying between- and within-individual variation for precision livestock farming. *Front Vet Sci.* (2022) 9:827124. doi: 10.3389/fvets.2022.827124
66. Knauer WA, Godden SM, Dietrich A, Hawkins DM, James RE. Evaluation of applying statistical process control techniques to daily average feeding behaviors to detect disease in automatically fed group-housed preweaned dairy calves. *J Dairy Sci.* (2018) 101:8135–45. doi: 10.3168/jds.2017-13947
67. Van De Gucht T, Saeys W, Van Nuffel A, Pluym L, Piccart K, Lauwers L, et al. Farmers' preferences for automatic lameness-detection systems in dairy cattle. *J Dairy Sci.* (2017) 100:5746–57. doi: 10.3168/jds.2016-12285

2.7 Limitations and further discussion

While the study above has added to the literature regarding associations between automatically recorded calf behaviours and intrinsic calf factors there are several key limitations that were not discussed in the paper. Firstly, only one teat per pen was available, this may have caused competition at the teat. In pair housed *ad libitum* fed calves the provision of one teat rather than two lowered milk consumption in the second week of life (Miller-Cushon *et al.*, 2014). This was however compensated for later in the milk feeding period. Competition is also increased in calves that are teat rather than bucket fed (Jensen and Budde, 2006). Interestingly, calves that experienced competition for milk feeding consumed more starter post- weaning (Miller-Cushon *et al.*, 2014).

Another factor that should be considered is the design of the milk feeder stall, some research suggests that steel bar stalls as used in this study lead to a delayed adaptation to the automatic milk feeder when compared to solid sided stalls (Wilson *et al.*, 2018). It was interesting that there was no association in this study between age at introduction to the group pen and any of the milk feeding behaviours as previous research has shown that calves introduced at an earlier age take longer to adapt to the automatic milk feeder e.g. Fujiwara *et al.* (2014), Jensen (2007) and Medrano-Galarza *et al.* (2018). The lack of an effect may be due to the small range in this variable (6 – 13 days) and as calves were included in the dataset 14.5 days following introduction to the pen on average (range: 3 – 23 days).

Another limitation is that the dataset was unbalanced and small (Table A1.1), there were only three dairy female calves in the dataset. Thus, it is exceedingly difficult to unpick the associations of the milk and activity variables with sex and sire-breed type. This limits the inferences that can be made from this study. This study was conducted on only one farm and there may therefore be factors influencing calf behaviour that do not occur in the same way elsewhere, e.g. temperature and wind speed. Further work with more calves that are better balanced across sex and sire-breed, involving multiple farms and levels of nutrition is required. In a larger data

set analysing the effect of individual sire-breeds would be advisable. Particularly as there were both native and continental sires in the beef category.

As shown above, both sex and sire-breed type are associated with calf behaviour. Sex can influence the response to social stress and traditionally males have been used when looking at social stressors (Proudfoot *et al.*, 2012). Differing responses to social stress in the group pen setting could account for some of the changes in behaviour seen in this study. With large dairy-beef companies rearing male and female calves of different breeds on automatic milk feeders, this may be an area of future commercial interest, and grouping calves according to breed and sex may aid in reducing competition and social stress. The findings around sex and breed may also influence the development of disease detection tools as they may need to use algorithms that include sex or breed to improve accuracy.

In conclusion, live-weight, age, sex and sire-breed type are all associated with calf behaviour. However, this study had several key limitations and this limits the inferences that can be drawn from its findings,

2.8 Bibliography

BUS, J. D., BOUMANS, I. J. M. M., WEBB, L. E. & BOKKERS, E. A. M. 2021. The potential of feeding patterns to assess generic welfare in growing-finishing pigs. *Applied Animal Behaviour Science*, 241, 105383.

CLARKE, H. 2024. How much beef is produced from the GB dairy herd? [Online]. <https://ahdb.org.uk/news/how-much-beef-is-produced-from-the-gb-dairy-herd>: Agriculture & Horticulture Development Board. Available: <https://ahdb.org.uk/news/how-much-beef-is-produced-from-the-gb-dairy-herd> [Accessed 10/03/2025 2025].

FUJIWARA, M., RUSHEN, J. & DE PASSILLÉ, A. M. 2014. Dairy calves' adaptation to group housing with automated feeders. *Applied Animal Behaviour Science*, 158, 1-7.

JENSEN, M. B. 2007. Age at introduction to the group affects dairy calves' use of a computer-controlled milk feeder. *Applied Animal Behaviour Science*, 107, 22-31.

JENSEN, M. B. & BUDDE, M. 2006. The Effects of Milk Feeding Method and Group Size on Feeding Behavior and Cross-Sucking in Group-Housed Dairy Calves. *Journal of Dairy Science*, 89, 4778-4783.

MEDRANO-GALARZA, C., LEBLANC, S. J., DEVRIES, T. J., JONES-BITTON, A., RUSHEN, J., MARIE DE PASSILLÉ, A., ENDRES, M. I. & HALEY, D. B. 2018. Effect of age of introduction to an automated milk feeder on calf learning and performance and labor requirements. *Journal of Dairy Science*, 101, 9371-9384.

MILLER-CUSHON, E. K., BERGERON, R., LESLIE, K. E., MASON, G. J. & DEVRIES, T. J. 2014. Competition during the milk-feeding stage influences the development of feeding behavior of pair-housed dairy calves. *Journal of Dairy Science*, 97, 6450-62.

PROUDFOOT, K. L., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2012. Linking the social environment to illness in farm animals. *Applied Animal Behaviour Science*, 138, 203-215

RUMINANT HEALTH & WELFARE GROUP 2025. GB Dairy Calf Strategy: A review of progress 2020-2023. <https://ruminanthw.org.uk/wp-content/uploads/2025/01/GB-dairy-calf-strategy-review-of-progress-Jan-2025-APP-compressed.pdf>.

SHARON, K. P., LIANG, Y., SANCHEZ, N. C. B., CARROLL, J. A., BROADWAY, P. R., DAVIS, E. M. & BALLOU, M. A. 2019. Pre-weaning plane of nutrition and Mannheimia haemolytica dose influence inflammatory responses to a bovine herpesvirus-1 and Mannheimia haemolytica challenge in post-weaning Holstein calves. *Journal of Dairy Science*, 102, 9082-9096.

WILSON, T. R., LEBLANC, S. J., DEVRIES, T. J. & HALEY, D. B. 2018. Effect of stall design on dairy calf transition to voluntary feeding on an automatic milk feeder after introduction to group housing. *Journal of Dairy Science*, 101, 5307-5316.

Chapter 3 The effect of bovine respiratory disease on feeding behaviours and activity variables in pre-weaned, group-housed, dairy-bred calves

3.0 Preface

In the previous chapter, the associations between intrinsic calf factors and healthy calf behaviour were explored. Age, live-weight, sex, and season were all associated with multiple milk feeding or activity variables. This information can now be used to inform an analysis of the associations between disease and calf behaviour. The objective of this chapter is to understand the association between bovine respiratory disease and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.

3.1 Abstract

Bovine respiratory disease (BRD) is a common disease in young calves. Addressing the challenges presented by BRD requires rapid identification of diseased calves, but disease identification is poor at present. A variety of scoring systems have been used to address this problem. However, these are time consuming. Technology provides the opportunity to monitor calf behaviour constantly thus reducing the labour requirement and removing any issues caused by diurnal variations.

Automatic milk feeders and tri-axial accelerometers are already present on many farms. Research suggests that behaviours measured by these technologies are associated with disease. The current study aimed to use a matched pair design to analyse the association between BRD and milk feeding and activity variables.

Group-housed dairy-bred calves were Wisconsin calf health scored daily from approximately seven days of age until weaning at thirty-nine days of age. Calves had access to seven litres of milk replacer daily and *ad-libitum* calf starter and straw.

Milk feeding behaviours were recorded using an automatic milk feeder and activity variables using a tri-axial accelerometer. From this data, eight activity variables and six milk feeding variables were calculated. Calves with BRD were paired with a healthy counterpart in the same pen, on the same date using sex, live-weight, and age. The day with the highest Wisconsin calf health score was identified, and three days either side of this day were extracted for analysis. Healthy counterparts could be classed as healthy for five days either side of its pair's day of peak BRD. Eighteen pairs were analysed for the milk feeding behaviours and fourteen pairs were analysed for the activity variables. Generalized linear mixed models were constructed to test BRD status, day relative to peak BRD day and the interaction between them as well as the calf factors live-weight, age, sex, sire-breed type, and age at inclusion into the group pen.

Calves with BRD visited the milk feeder for longer on the three days following peak BRD and drank more milk at each visit on days -3 and -2 relative to peak BRD. Both mean milk drinking speed and total time at milk feeder had an interaction between BRD and day versus peak BRD, but no pairwise comparisons between BRD and healthy calves on individual days were significant. There was no association

between the activity variables and BRD, day relative to peak BRD or the interaction between them. Heavier calves had shorter but more frequent visits to the milk feeder, drinking less at each visit but were more likely to drink their daily allowance. Results in this study differ from some of the existing research. This may be due to differences in study design, including statistical analysis, definitions of disease, calf nutrition, and sample size. Overall, calves on a lower plane of nutrition appear to show fewer changes in behaviour with disease than those on a higher plane of nutrition.

3.2 Introduction

Bovine respiratory disease (BRD) is a common disease in calves. The disease is multifactorial (Closs and Dechow, 2017) and frequently involves both viral and bacterial pathogens (Workman *et al.*, 2019). Respiratory disease is considered as having a high level of welfare consequence (EFSA Panel on Animal Health and Animal Welfare, 2023). Calves are particularly likely to suffer if disease leads to pain or respiratory distress (Mellor and Stafford, 2004). Disease also affects mortality, growth and long-term reproduction and milk production (see section 1.2). Thus, disease needs to be identified promptly to maintain animal welfare and production. Prompt treatment with antibiotics has been shown to reduce the need for re-treatment, and increase weight gain (Binversie *et al.*, 2020). Despite the need for early, accurate identification of diseased animals previous research suggests that the identification of BRD on farm is poor (Olson *et al.*, 2019, McConnel *et al.*, 2019, Baxter-Smith *et al.*, 2022). Furthermore, research suggests that clinical signs may vary throughout the day (Lowie *et al.*, 2024). Thus, using technologies that can record calves throughout the day may be beneficial compared to intermittent manual assessments e.g. once daily health scoring. Therefore, there are opportunities to utilise technology to allow us to detect disease.

Automatic milk feeders may already be utilised on farm to reduce the labour requirements of feeding calves (Sinnott *et al.*, 2021) and many farms use accelerometers in their adult cows (Borchers *et al.*, 2016). The current research shows a variety of findings on the association between disease and behaviour

variables. In one study, calves with BRD had a lower drinking speed, but did not change their milk intake (Conboy *et al.*, 2021). Another study found that milk intake did reduce, but not drinking speed, as well as there being changes in lying time, lying bout and activity (Cantor and Costa, 2022). This is further explored in section 1.6.1.

The challenges of identifying BRD for research have been extensively discussed in section 1.4.3 of this thesis. For this study, the Wisconsin Calf Health Score (WCHS) was chosen as the diagnostic method for BRD. The reasoning behind this was that it provides better granularity than the Californian scoring system, the only equipment required was a thermometer and it requires calves to be restrained for much less time than TUS. This allows daily scoring without overly affecting the calves. Daily monitoring was needed so that identification of disease was prompt. In addition, TUS will not detect upper respiratory tract disease or disease away from the pleural surface. In contrast as the WCHS includes assessing ocular and nasal discharge and ear droop it does consider upper respiratory tract disease.

The objective of this chapter was to understand the association between bovine respiratory disease and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves. The results of Chapter 2 (Riley *et al.*, 2023) will be used to inform the matched pair analysis of the activity and milk feeding variables.

3.3 Methods

3.3.1 Animal management

Calves for this study were sourced from two herds (herds A and B) within the SRUC Dairy and Innovation Research Centre, Crichton Royal Farm, Dumfries. The experiment was reviewed by SRUC's animal experiments committee (Experiment ID: DAI AE 10-2020). Calves born between 24.07.20 and 11.01.21 were included in the trial. Due to a long-term trial examining genetic and environmental interactions, calves from herd A came from two distinct Holstein lines: a UK-average yield line and a high yielding line. Only male Holstein calves were included from this herd. All

dams were Holstein. The sire breeds of the 114 calves recruited onto the trial are shown in Table 3.1.

Table 3.1 Sex, herd of origin, genetic line and breeds of calves recruited onto the trial

Sire-breed type	Sire breed	Herd	Dam Genetic Line	Sex		Total
				Male	Female	
Beef	Aberdeen Angus	A	High	-	1	46
		B	UK average	-	1	
	Belgian Blue	A	High	3	3	-
		B	UK average	-	-	1
	Longhorn	A	High	17	8	-
		B	UK average	-	-	-
		B	UK average	5	7	-
	Dairy	Holstein	A	High	25	-
B			UK average	15	-	
B			UK average	2	26	-
Total				67	47	114

All calves received four litres of pasteurised, quality checked, thawed colostrum by stomach tube as soon as possible after birth. As is normal practice for this farm, calves were removed from their dam in the first twenty-four hours of life and placed in individual straw bedded hutches. Here, calves received three litres of reconstituted acidified milk replacer in a bucket with a teat twice daily (*Maximum +, Carrs Billington Ltd.*, crude protein 24%, crude oils and fats 20%, crude ash 7.5%, calcium 0.9%, sodium 0.5%, phosphorus 0.7%, mixed to 15%). Starter pellets (compounded for the farm by *Forfarmers UK Ltd. Rougham*, dry matter 82.2%, crude protein 18%, crude oils and fats 4.5%, crude fibre 10%, crude ash 10.5%, Calcium 2%, decoquinate (*Deccox 6%, Zoetis UK Ltd. Leatherhead Surrey, 50 mg/kg*)) and water were offered in buckets *ad libitum*, from entering the hutch. On the advice of the farm's veterinary surgeon, all calves born between 24.07.20 and 31.12.20 received halofuginone (*Halocur 0.5 mg/ml oral solution for calves, MSD Animal Health, Walton, Milton Keynes, 100 µg/kg live-weight by mouth*) once daily from 0-6 days of age. For calves born after 01.01.21, this was changed to paromomycin sulphate given in milk between days zero and six of life (*Paroform, Huvepharma NV, Antwerp, Belgium, 10 g/calf/day*).

Calves were moved into groups of twelve to fourteen calves and entered the study at six to thirteen days of age (mean 8.1 days, median 8 days), data was used from the midnight following the calf entering the group pen. Calves eligible for the trial

would be added to the group pen on a weekday and calves continued to be added to a pen until it was full. Calves were not moved into an empty pen on their own. The time to fill a group pen was 13.4 days on average (6 – 29 days, median: 13 days). Group pens were straw bedded and consisted of a roofed pen (5.1 m x 5.1 m) and an igloo (*Holm & Laue, Moorweg 6, 24784 Westerrönhof, Germany, 3.9 m, 4.4 m, 2.2 m*) (stocking density 3.1 - 3.6 m²/calf). At time of entry to the group pen, a tri-axis accelerometer was placed on the right hind leg (*IceQube, IceRobotics, Edinburgh*). Calves were offered *ad libitum* calf starter (as above) in a trough and straw in a hay rack. Each group pen had a single automatic calf feeding unit (*Custom built for this calf unit, BioControl Norway As, Grimstad Gård, Norway*) which consisted of a milk lane and a water lane for each pen (1 teat for 12-14 calves). Milk was mixed as above and calves were allowed seven litres between 00:00 and 23:59. If they had not reached this maximum, they could carry over 1.4 litres into the next day. Water was available *ad libitum*. Both water and milk lanes included a weigh-scale and a live-weight measurement was recorded at each visit. In addition, calves were weighed manually at entry to the pen, at 28 days of age and again after weaning.

In order to identify BRD the WCHS was carried out as described by McGuirk (2015) daily by one of three trained technicians. As neonatal calf diarrhoea was not the subject of this study and faeces in a group pen cannot be attributed to one animal the faeces score element of WCHS was disregarded for this study. The WCHS is shown in Figure 3.1. To carry out scoring, all calves were closed into the front of the pen for the period during which scoring took place, approximately fifteen to forty-five minutes. A daily total WCHS for each calf was calculated using the rectal temperature, cough, and nasal discharge scores and whichever was highest of either the ear or eye scores. Veterinary treatments were administered by the farm staff according to existing protocols independently of the WCHS.










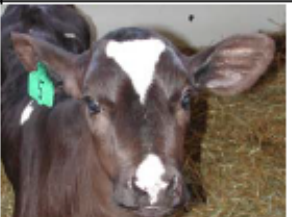


Calf Health Scoring Criteria			
0	1	2	3
Rectal temperature			
100-100.9	101-101.9	102-102.9	≥103
Cough			
None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge			
Normal serous discharge	Small amount of unilateral cloudy discharge	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
			

Figure 3.1 Wisconsin calf health scoring system (McGuirk, 2015)

3.3.2 Data handling

Data was exported into Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA) for initial data handling. To ensure only complete days were available for analysis, the day a calf moved into the group pen and any days where calves were moved into individual pens for treatment were removed. Activity data where there were missing segments of the day was also removed. Data was included from the day following a calf's entry into the group pen until the day prior to weaning (thirty-nine days of age). The time frame (7:15 - 9:15) during which the

majority (90%) of scoring took place was removed from the activity data as the technicians moved calves around during scoring.

R (R Core Team, 2021) was used for data processing and analysis using the R Studio graphical interface (*R studio, Boston, Massachusetts*). The tidyverse package (Wickham *et al.*, 2019) was used to process the data into daily values for each calf. The output from the tri-axis accelerometer was processed into daily lying time (minutes), daily standing time (minutes), daily lying bouts (n), daily standing bouts (n), total daily motion index, mean motion index per standing bout, mean lying bout length (minutes), and mean standing bout length (minutes). The data from the automatic milk feeder was processed into total time at milk feeder (minutes), total milk visits (n), mean milk visit length (minutes), mean milk drinking speed (g/s), volume of milk drunk/day (ml) and mean milk per visit (ml). The psych package (Revelle, 2021) was used to calculate descriptive statistics. The live-weight values were additionally cleaned by removing any values greater than five kilograms away from the next largest/smallest and any values greater than two standard deviations from the mean.

One hundred and fourteen calves were recruited onto the trial, of these, seven calves were excluded from further analysis. Reasons for exclusion were reluctant to drink (n = 1), mortality due to bloat (n = 1), congenital heart defect (n = 1), ear tag infection/iatrogenic ear droop (n = 3) and a faulty IceQube (n = 1). A further nine calves did not stay in the group pen until weaning for reasons including mortality (n = 3), removed to receive nursing care (n = 4), lameness (n = 1), and ear swelling (n = 1). Equipment problems or calves not being in close enough proximity to the base station led to eight calves having missing activity data. Technical problems meant that three days of automatic milk feeder data was missing for all calves.

To perform the matched pair analysis, BRD calves with a peak WCHS ≥ 5 were paired with a healthy calf in the same group, of the same sex, within seven days of age and within ten kilograms of live-weight. These pairing criteria were chosen based on the findings of Chapter 2 (Riley *et al.*, 2023). Healthy calves had no treatment events, a rectal temperature of $< 39.4^{\circ}\text{C}$ and a WCHS of ≤ 3 for five days prior to and after the peak BRD day of its pair. Eighteen pairs were analysed for the

feeding behaviours. Due to missing days of data due to faulty equipment or calves not spending sufficient time in proximity to the base station, four calf pairs had missing activity data, thus fourteen pairs were included in the analysis of activity behaviours. To check that the healthy and BRD groups were balanced general linear mixed model was built for each of the continuous explanatory variables, age, live-weight, birthweight, and age at inclusion into the group pen. Each model contained disease, day relative to peak disease and the interaction between them as fixed effects and calf identity as a random effect.

3.3.3 Data analysis

General linear mixed modelling using the lmer function in the lme4 package (Bates *et al.*, 2015) of R was performed to assess the effect of BRD, day relative to peak BRD and the interaction between them. Calf identity was included as a random effect. The activity variables tested were daily lying time, daily standing time, daily lying bouts, daily standing bouts, mean lying bout length, mean standing bout length, total daily motion index, and mean activity per standing bout. The milk feeding variables tested were total time at milk feeder, total milk visits, mean milk visit length, mean milk per visit and mean milk drinking speed.

Two separate maximal models were constructed, one containing live-weight and another age (live-weight and age were highly correlated in this study) and backwards model selection was conducted using the step function in the lmerTest package (Kuznetsova *et al.*, 2017). Age at inclusion into the group pen, sire-breed type (dairy or beef, Table 3.1) and sex, and the random effect of calf number were included in both maximal models. If both age and live-weight were selected in the model selection, the Akaike information criterion corrected for small sample sizes (AICc, aictab, AICcmodavg, (Mazerolle, 2020)), was calculated and the model with the smallest AICc was used as the final model. Where the backward model selection resulted in the variables of interest: disease status, day relative to peak disease and the interaction terms being removed they were re-added to the final model. The summary() function in the lmerTest package (Kuznetsova *et al.*, 2017) was used to calculate the estimates, t values, and p values from the model, and the confint() function (Bates *et al.*, 2015) was used to calculate the confidence intervals of the

estimates. The numerator degrees of freedom and denominator degrees of freedom were calculated using the `anova()` function (R Core Team, 2021). Residuals were assessed using the `simulateResiduals` and `plot` functions in the DHARMA package (Hartig, 2022). If necessary, a transformation was used on the dependant variable; total time at milk feeder and mean milk visit length were analysed using a natural log transformation. Estimated marginal means and their associated standard errors were calculated using the `emmean` function in the `emmeans` package (Lenth, 2024) and pairwise comparisons were used between the BRD and healthy calves for each day relative to peak BRD. Line graphs of the estimated marginal means were produced using `ggplot` (Wickham, 2016).

Due to the calves in this study being limit fed, there was a left skew in the total milk drunk/day data, thus it was not suitable for analysis using a Gaussian method. This data was analysed using a binomial generalised linear mixed model (`glmer`, `lme4`, (Bates *et al.*, 2015)). Those calves that had drunk more than 6.5 litres of milk in the day were coded as 1 and those that had drunk less than 6.5 litres were coded as 0. Age at inclusion into the group pen, sire-breed type (dairy or beef), sex, live-weight and age were each modelled separately alongside the variables of interest; BRD, day relative to BRD and their interaction term. Models were visualised using the `summary()` function in `lmerTest` (Kuznetsova *et al.*, 2017). Forward model building (as `step()` is not compatible with `glmer()`) was carried out using those variables with $p < 0.1$ and the variables of interest. All models included calf identity as a random effect. The Akaike information criterion corrected for small sample sizes, was calculated (`AICc`, `aictab`, `AICcmmodavg`, (Mazerolle, 2020)) and the model with the smallest value was selected as the final model. Overdispersion was checked for using the equation suggested by Bolker (Bolker, 2021). The odds ratio and confidence intervals were then calculated using base R (R Core Team, 2021) and `lme4` (Bates *et al.*, 2015). The Z and p values were calculated using the `summary()` function in `lmerTest` (Kuznetsova *et al.*, 2017).

3.4 Results

In this study, calves were paired to create a balanced dataset. Table 3.2 demonstrates that the pairs were well matched with no significant difference ($p > 0.05$) in age, live-weight, age at inclusion or birthweight between the two groups. The sire-breed type and sex for the matched pairs are displayed in Table 3.3. The descriptive statistics of each behaviour are presented according to their disease status in Table 3.4.

Firstly, in this section the results for the main variables of interest, disease status, day relative to peak BRD and the interaction between them will be presented followed by the other factors found in the final models.

Table 3.2 Raw means and standard error of the means (SEM) for bovine respiratory disease (BRD) and healthy calves and p values calculated from a mixed model for each continuous explanatory variable.

Calf factor	Healthy		BRD		P value
	Mean	SEM	Mean	SEM	
Age (days)	25.3	0.7	25.2	0.7	0.904
Live-weight (kg)	42.5	0.6	55.1	0.6	0.504
Age at inclusion into the group pen (days)	8.8	0.2	8.2	0.2	0.133
Birthweight (kg)	53.6	0.5	44.5	0.4	0.729

Table 3.3 The sex and sire-breed type of the calves included in the final analyses.

Sire-breed type	Sex	Milk feeding variables		Activity variables	
		Healthy	BRD	Healthy	BRD
Beef	Male	2	6	2	6
	Female	2	2	2	2
Dairy	Male	10	6	8	4
	Female	4	4	2	2
Total		18	18	14	14

Table 3.44 Descriptive statistics of activity and milk feeding variables in paired calves according to disease status

	Healthy					Bovine respiratory disease				
	Mean	SD	Minimum	Maximum	SEM	Mean	SD	Minimum	Maximum	SEM
Daily lying time (minutes)	962.6	74.7	681.7	1152.4	6.9	961.8	73.0	677.2	1130.3	6.6
Daily standing time (minutes)	357.3	74.8	167.6	638.3	6.9	358.2	73.0	189.7	642.8	6.6
Daily lying bouts (n)	18.9	5.7	9.0	34.0	0.5	17.2	5.0	8.0	33.0	0.4
Daily standing bouts (n)	19.0	5.6	9.0	35.0	0.5	17.2	4.9	9.0	33.0	0.4
Total daily motion index	3384.9	1516.2	1020.0	7334.0	140.8	2982.8	1564.1	456.0	8043.0	140.5
Mean lying bout length (minutes)	55.3	16.0	21.8	92.3	1.5	60.4	18.0	27.8	130.8	1.6
Mean standing bout length (minutes)	20.6	8.7	8.9	63.8	0.8	22.3	6.9	7.0	50.7	0.6
Mean motion index per standing bout	182.6	74.3	65.1	408.9	6.9	177.9	88.9	21.7	485.5	8.0
Total time at milk feeder (minutes)	15.8	4.1	5.3	35.6	0.4	16.1	4.0	7.8	35.5	0.4
Total milk visits (n)	8.6	2.5	2.0	16.0	0.2	8.1	2.1	3.0	15.0	0.2
Mean milk visit length (minutes)	1.8	0.5	1.2	3.3	0.0	2.0	0.6	1.1	4.4	0.1
Mean milk drinking speed (g/s)	7.0	1.2	2.8	9.8	0.1	6.8	1.4	2.6	11.1	0.1
Daily milk volume (ml)	6643.7	922.9	1882.0	8416.0	82.2	6623.0	826.6	3364.0	7996.0	73.6
Mean milk per visit (ml)	800.8	224.2	345.2	1327.6	20.0	849.5	223.1	399.3	1361.8	19.9

3.4.1 The association between bovine respiratory disease and activity and milk feeding variables.

From the six milk feeding behaviours and eight activity variables analysed, only total time at the milk feeder, mean milk feeder visit length, mean milk per visit and mean milk drinking speed were associated with BRD, day relative to peak BRD or the interaction between them. In the final model, calves with BRD tended to consume more milk and spend longer at each visit to the feeder ($p < 0.090$ and $p < 0.061$ respectively, Table 3.5). When pairwise analysis of estimated marginal means were performed, calves with BRD had longer visits to milk on the three days after peak BRD ($p < 0.05$, Figure 3.2A) and drank more milk at each feeder visit on days -3 and -2 relative to peak BRD ($p < 0.05$, Figure 3.2B). Mean milk drinking speed showed an interaction between BRD and day relative to peak BRD in the final model output ($p = 0.015$, Table 3.5). In the pairwise contrasts between healthy and BRD calves for each day relative to peak BRD, only a tendency for a reduction in drinking speed on day 3 was seen ($p = 0.057$, Figure 3.2C). The final model output for total time at the milk feeder also showed an interaction between BRD and day relative to peak day of BRD ($p = 0.049$, Table 3.5), however, no pairwise comparisons between the estimated marginal means of BRD and healthy calves were significant on any day relative to peak BRD ($p > 0.1$, Figure 3.2D). There was no association between BRD, day relative to peak BRD or the interaction between them for any of the activity variables (Table 3.6 - Table 3.7).

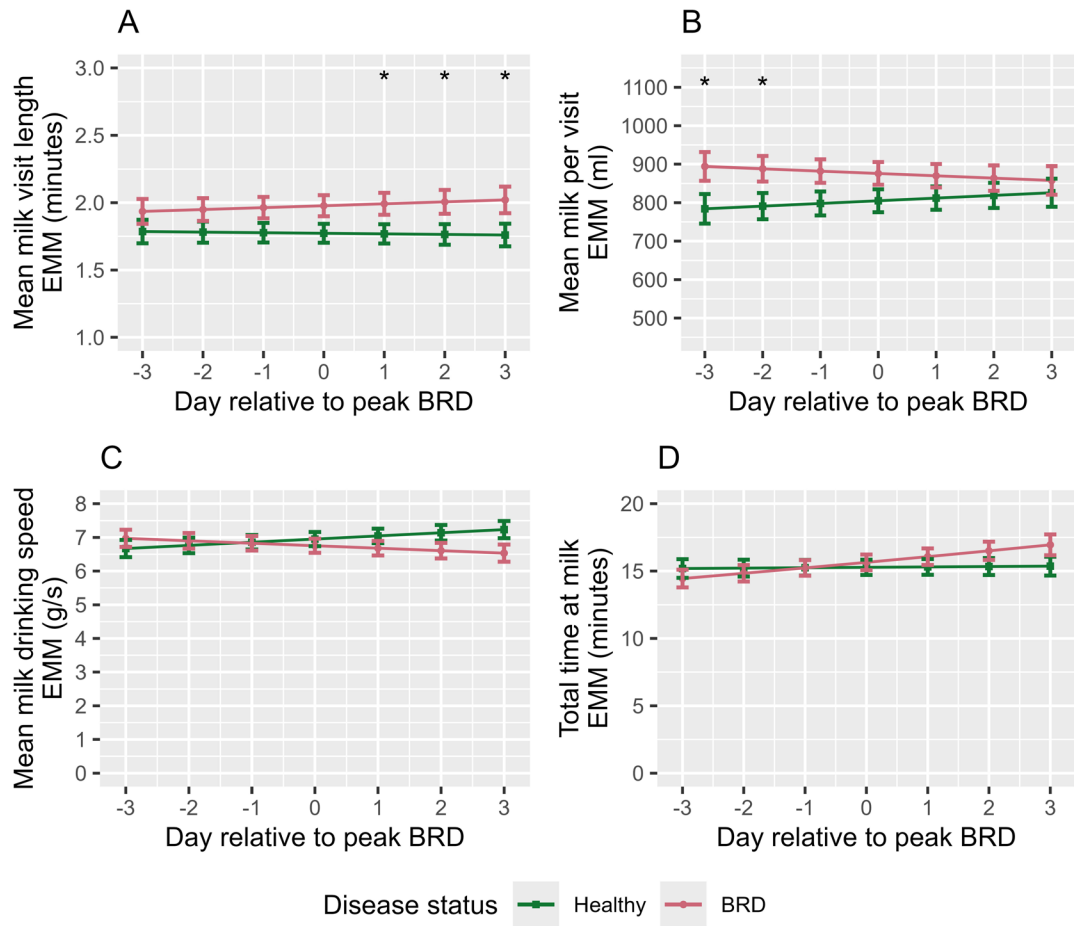


Figure 3.2 The association between bovine respiratory disease (BRD) and day relative to BRD and (A) mean milk visit length, (B) mean milk per visit, (C) mean milk drinking speed and (D) total time at milk in eighteen pairs of pre-weaned calves, as illustrated by the estimated marginal means (EMM) calculated from the final model. Asterisks represent a significant difference ($p < 0.05$) between diseased and healthy calves in a pairwise comparison. Error bars represent the standard error.

Table 3.5 The association between bovine respiratory disease (BRD) and day relative to peak BRD and milk feeding variables in eighteen pairs of pre-weaned calves. The results shown are from the final linear models^a. Variables shown in bold have $p < 0.05$.

Variables	Fixed effect	Level	Effect size	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t value	P value
Total time at milk feeder (minutes) ^a	Disease status	Healthy	Reference	Reference	1	34	Reference	Reference
		BRD	1.02	-0.92 – 1.14			0.454	0.653
	Day		1.00	-1.02 – 1.05	1	214	0.218	0.828
	Disease status x day							0.049
Total milk visits (n)	Disease status	Healthy	Reference	Reference	1	33.226	Reference	Reference
		BRD	-0.71	-1.66 -0.24			-1.441	0.159
	Day		0.07	-0.23 – 0.08	1	245.345	-0.907	0.366
	Live-weight		0.14	0.07 – 0.21	1	39.740	4.010	< 0.001
	Disease status x day							0.406
Mean milk visit length (minutes) ^a	Disease status	Healthy	Reference	Reference	1	32.603	Reference	Reference
		BRD	1.12	1.00 - 1.24			-1.943	0.061
	Day		-1.00	-1.02 – 1.02	1	245.520	-0.263	0.793
	Live-weight		-1.02	-1.02 – -1.01	1	38.905	-4.087	< 0.001
	Disease status x day							0.438
Mean milk per visit (ml)	Disease status	Healthy	Reference	Reference	1	31.962	Reference	Reference
		BRD	71.05	-6.06 – 148.17			1.751	0.090
	Day		6.98	-7.91 – 21.70	1	245.355	0.920	0.359
	Sex	Female	Reference	Reference	1	32.557	Reference	Reference
		Male		-92.03	-179.31 – 4.75			-2.005
	Live-weight		-14.61	20.64 - -8.56	1	37.401	-4.617	< 0.001
	Disease status x day							0.205
Mean milk drinking speed (g/s)	Disease status	Healthy	Reference	Reference	1	34	Reference	Reference
		BRD	-0.20	-0.78 – 0.38			-0.665	0.510
	Day		0.09	-0.00 – 0.19	1	214	1.942	0.053
	Disease status x day							0.015

^aThe results shown for the models where a transformation was used have been back transformed.

Table 3.6 The association between bovine respiratory disease (BRD) and day relative to peak disease and lying and standing variables in fourteen pairs of pre-weaned calves. The results shown are from the final linear models.

Variables	Fixed effect	Level	Effect size	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t value	P value
Daily lying time (minutes)	Disease status	Healthy BRD	Reference	Reference	1	26	Reference	Reference
	Day		-3.28	-34.76 - 28.21	1	166	-0.204	0.840
	Disease status x day		2.31	-4.32 - 8.95	1	166	0.684	0.495
Daily lying bouts (n)	Disease status	Healthy BRD	Reference	Reference	1	26	Reference	Reference
	Day		-0.95	-3.56 - 1.67	1	166	-0.710	0.484
	Disease status x day		0.02	-0.43 - 0.46	1	166	0.078	0.938
Mean lying bout length (minutes)	Disease status	Healthy BRD	Reference	Reference	1	26	Reference	Reference
	Day		2.21	-5.98 - 10.41	1	166	0.528	0.602
	Disease status x day		0.22	-1.24 - 1.69	1	166	0.299	0.765
Daily standing time (minutes)	Disease status	Healthy BRD	Reference	Reference	1	26	Reference	Reference
	Day		3.43	-28.08 - 34.94	1	166	0.213	0.833
	Disease status x day		-2.43	-9.06 - 4.21	1	166	-0.717	0.474
Daily standing bouts (n)	Disease status	Healthy BRD	Reference	Reference	1	26	Reference	Reference
	Day		-1.07	-3.67 - 1.53	1	166	-0.806	0.428
	Disease status x day		-0.05	-0.49 - 0.40	1	166	-0.203	0.839
Mean standing bout length (minutes)	Disease status	Healthy BRD	Reference	Reference	1	26	Reference	Reference
	Day		0.66	-3.29 - 4.61	1	166	0.328	0.746
	Disease status x day		0.06	-0.58 - 0.69	1	166	0.176	0.860
								0.961

Table 3.7 The association between bovine respiratory disease (BRD) and day relative to peak disease and activity variables in fourteen pairs of pre-weaned calves. The results shown are from the final linear models.

Variables	Fixed effect	Level	Effect size	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t value	P value
Total daily motion index	Disease status	Healthy	Reference	Reference	1	25	Reference	Reference
		BRD	-266.63	-1023.60 – 490.33			-0.675	0.506
	Day		-63.70	-198.03 – 70.63	1	168.25	-0.922	0.358
	Age		-35.14	-85.78 – 15.49	1	25	-1.331	0.195
		Disease status x day						0.699
Mean motion index per standing bout	Disease status	Healthy	Reference	Reference	1	26	Reference	Reference
		BRD	-5.64	-41.07 – 29.79			-0.311	0.758
	Day		-4.51	-11.61 – 2.59	1	166	-1.244	0.215
		Disease status x day						0.842

Table 3.8 The effect bovine respiratory disease (BRD) and day relative to peak disease and other factors had on the odds ratio of pre-weaned calves having drunk their daily total milk allowance. Variables shown in bold have $p < 0.05$.

Fixed effect	Level	Odds Ratio	Confidence interval	Z value	P value
Disease status	Healthy	Reference	Reference	Reference	Reference
	BRD	0.63	0.16 – 2.55	-0.641	0.521
Day		1.08	0.81 – 1.43	0.522	0.601
Live-weight		1.13	1.01 – 1.26	2.130	0.033
Disease status x day					0.823

3.4.2 The association between milk feeding, activity variables and intrinsic calf factors.

Heavier calves visited the milk more frequently ($p = 0.006$, Figure 3.3A), for a shorter time ($p = 0.005$, Figure 3.3B) and drank less milk per visit ($p = 0.001$, Figure 3.3C) but were more likely to drink their daily milk allowance ($p = 0.033$, Figure 3.3D, Table 3.8). Sex was included in the model for mean milk per visit as male calves tended to have a smaller feed at each visit ($p = 0.053$, Table 3.5). Age was included in the model for total daily motion index as it improved model fit ($p = 0.195$, Table 3.7).

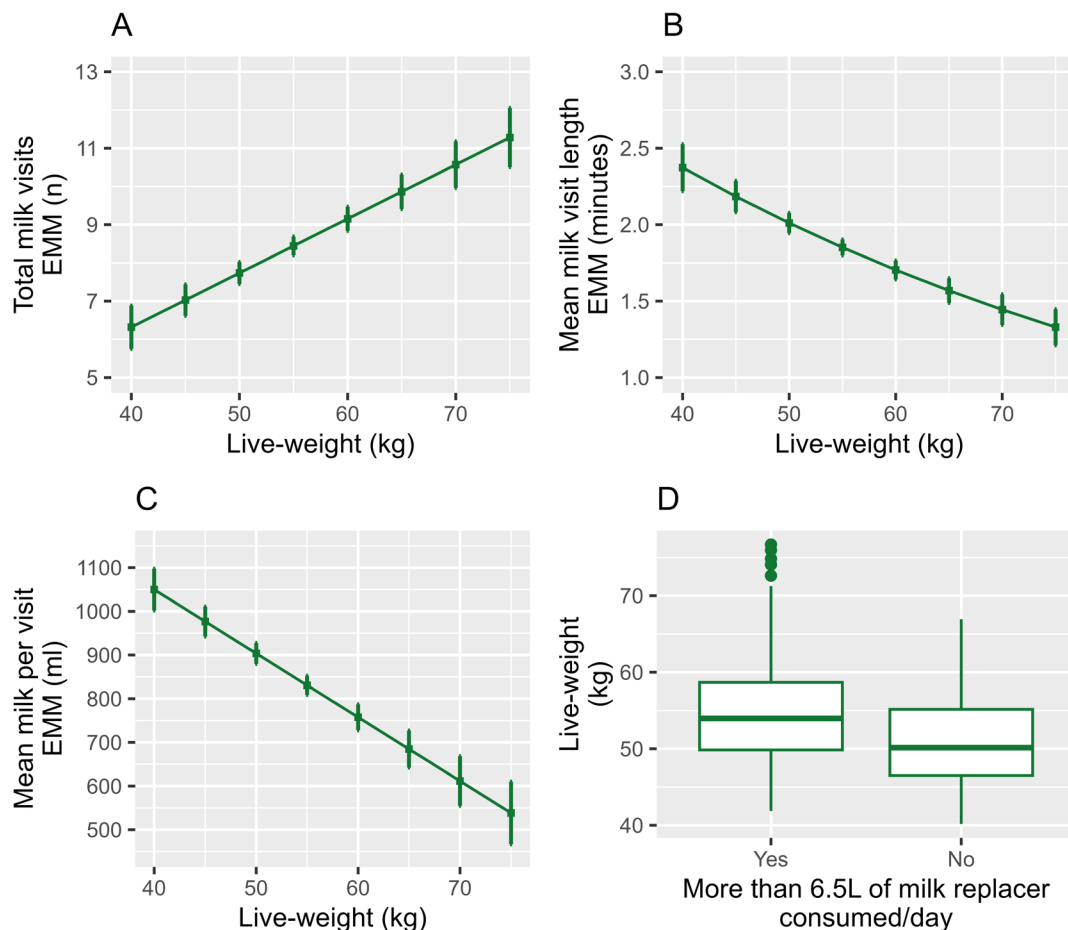


Figure 3.3 The association between live-weight and A) total milk visits, B) mean milk visit length and C) mean milk per visit in eighteen pairs of pre-weaned calves as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars represent the standard error. D) A boxplot showing the live-weight of calves that had and had not consumed their daily milk allowance

3.5 Discussion

This study aimed to examine the associations between bovine respiratory disease (BRD) and milk feeding behaviours and activity variables. Calves visited the milk for longer after BRD and drank more milk at each visit before BRD. Heavier calves visited the milk more frequently for less time, drank less milk at each visit and were more likely to drink their daily allowance.

The findings in this study were slightly different to those of previous studies. This may be due to different subtypes of pneumonia occurring, or the disease definition used in the study. Cantor and Costa (2022) only included clinical pneumonia in their study, which they defined as having a WCHS ≥ 5 and lung consolidation of > 3 cm, they found that in calves with BRD, milk intake, lying bouts, and total daily motion index decreased, and lying time increased. In calves that received a tracheal inoculation of the bacterial pathogen *Mannheimia haemolytica*, feeding time decreased and lying time increased compared to control calves (Hixson *et al.*, 2018). In a study using the WCHS, as used in the current study, it was found that calves with BRD had a lower drinking speed and fewer unrewarded visits than their healthy counterparts (Conboy *et al.*, 2021). A study looking at slightly older calves (21 ± 7 - 50 days of age) defined disease as having two WCHS categories scoring two or more (Figure 3.1) and lung consolidation (Cramer *et al.*, 2020). The authors found that calves with BRD drank slower than calves with sub-clinical BRD (lung consolidation but no clinical signs) or no BRD. However, BRD had no effect on milk intake, average meal size, or number of rewarded or unrewarded visits.

Interestingly, in calves with BRD as defined by both a WCHS and lung consolidation, calves that had not recovered seven days after treatment had a slower drinking speed, reduced milk intake and fewer lying bouts in the seven days after treatment when compared to calves that recovered in that time (Cantor *et al.*, 2022). It should also be considered that the results of any diagnostic, including both clinical scoring and TUS, will vary depending on the pathogen responsible for disease (Baruch *et al.*, 2019).

Another possible reason for the differences between studies is the calves' milk allowance. Borderas *et al.* (2009) found that in calves allowed twelve litres a day, or

ad libitum access to milk, the daily milk intake and number of visits to milk decreased and the milk visit length increased in BRD calves compared to their healthy counterparts. However, in calves who only have access to four litres of milk a day, the only change in feeding behaviours seen was a reduction in the milk visit length (Borderas *et al.*, 2009). These findings were supported by Lowe *et al.* (2021), calves fed either five or ten litres a day both increased their visits to milk in the days prior to disease but only calves on the higher nutritional plane had an increase in the percentage of visits that were rewarded or the percentage of milk allowance consumed. Prior to disease development both groups had fewer, longer lying bouts. However, the calves on five litres of milk a day lay down for less time each day and the calves on ten litres of milk a day lay down for longer each day. Similarly, Johnston *et al.* (2016) found that there was an interaction between disease status and plane of nutrition, both prior to disease detection and on the day BRD was detected. Furthermore, Perttu *et al.* (2023) found that there was a complex relationship between drinking speed and milk intake. In general, an increased drinking speed was associated with a lower disease risk, however if the milk allocation was over seven litres, then a slow drinking speed was associated with a decreased milk intake. It is worth noting that no consideration of age was made for this interaction, despite the calves in the study being on a feeding curve dependant on age.

These differences may be reflective of differences in the immune response. Calves fed on a higher level of milk replacer have a reduction in several lines of T cells and reduced expression of several T cell surface proteins (Foote *et al.*, 2005b). A decrease in interferon gamma and increase in nitric oxide secretion from white blood cells has been seen in calves fed and intensified diet (Foote *et al.*, 2005a). Griebel *et al.* (1987) found a reduction in interleukin-2, lymphocyte proliferation, and antibody response to being inoculated with an antigen in calves that received less milk. Obeidat *et al.* (2013) found an increase in neutrophil L-selectin and neutrophil oxidative burst in calves fed less milk. A reduction in immune function in calves fed less milk remains after weaning. Calves on a lower plane of nutrition pre-weaning showed a more severe pathophysiological response to Bovine Herpes

Virus and *M. haemolytica* challenge post-weaning, with higher white blood cell counts and levels of inflammatory markers (Sharon *et al.*, 2019). Calves fed on a higher level of milk have greater plasma haptoglobin following challenge with lipopolysaccharide and their white blood cells respond differently to *ex vivo* challenge seventy-seven days later (Ballou, 2012). These changes in immune function may explain previous findings that environmental factors influence the expression of sickness behaviours (Sylvia and Demas, 2017).

While some differences may be due to factors unique to the farm a study performed on the same farm as the current study where calves were fed *ad libitum* found that mean milk visit length was longer on days -3, 1 and 3 relative to peak BRD in calves with BRD, while in this study, mean milk visit length was longer on days 1, 2 and 3 relative to peak BRD in calves with BRD. Unlike in this study, the previous study found no association between BRD and milk consumed at each visit and did find an association with number of visits to milk, total time at milk, daily volume of milk consumed, daily lying time and daily lying bouts (Duthie *et al.*, 2021). However, the previous study had twenty-two calf pairs, so these differences may also be due to reduced power in the current study.

In a recent study examining different machine learning models for BRD detection, walking distance was identified as a key feature (Bushby *et al.*, 2024). Motion index is the closest thing to walking distance in this study, as it is a measure of how active the calf is; however, it was not associated with BRD in this study.

There are challenges in comparing studies, even between those using matched paired designs. In the current study there were eighteen calf pairs analysed for the milk feeding behaviours and fourteen pairs analysed for the activity variables and the day of peak BRD was day 0 in the model, similarly Duthie *et al.* (2021) used day of peak BRD as day 0 and had twenty-two matched pairs. Knauer *et al.* (2017) used day of treatment as day 0 in a study with fifty-three matched pairs and found that drinking speed and unrewarded visits were lower in BRD calves on the day of treatment, but there was no differences ten days either side or in rewarded visits or milk consumed each day. In a study with ten matched pairs, Swartz *et al.* (2017) defined day 0 as the first day the WCHS was more than four, in that study BRD

calves had fewer lying bouts on days -2,-1, 1 and 3, relative to the first BRD day and had a lower milk intake on day 0 relative to first BRD day but no differences in drinking speed, rewarded visits, unrewarded visits or lying time was seen. Thus, even in studies using similar designs, differences in the definition of day 0, in the behaviours measured and in the power of the studies means that direct comparisons are not possible.

A weakness of this study is that the feeders used did not record unrewarded visits. Several studies have found an association between unrewarded visits and disease. In a study with a matched pair design, calves with BRD had fewer unrewarded visits to the milk feeder prior to disease identification compared to healthy calves (Johnston *et al.*, 2016). Conboy *et al.* (2021) and Mahendran *et al.* (2023) also found that calves with BRD had fewer unrewarded visits to the milk feeder than their healthy counterparts.

A variety of modelling techniques have been used to predict disease. Where three cumulative sum statistical processing charts have been used in parallel for mean daily drinking speed, total daily milk consumption and daily number of unrewarded visits, an accuracy of 59.1%, a sensitivity of 74.9% and a specificity of 27.1% was achieved (Knauer *et al.*, 2018). Bowen *et al.* (2021) used a combination of a random forest machine learning model incorporating both activity and feeding variables and deviation from moving averages of lying time to predict BRD. This provided a specificity of 95%, a sensitivity of 54%, and an accuracy of 75%. Thus, while these techniques show promise, an improvement in the accuracy is required.

In this study, heavier calves had more frequent, shorter visits to the milk feeder and drank less at each visit but were more likely to consume their milk allowance. In Chapter 2 (Riley *et al.*, 2023), heavier calves also visited the milk more often for shorter visits and were more likely to consume their milk allowance. However, birthweight was in the final model for mean milk per feed and not live-weight. In the current study, birthweight was not analysed, as it was missing for one of the healthy calves. The lighter calves having fewer visits to the feeder and drinking more at each visit may reflect them feeling a level of competition. Dry cows sharing a feed trough had fewer larger meals than those that had their own trough

(Hosseinkhani *et al.*, 2008). Calves fed on an automatic milk feeder may modify their behaviour due to competition (Montes *et al.*, 2023). More dominant calves will disturb other calves when drinking (Stephens, 1974). Social standing may be associated with the rate of weight gain in calves (Broom and Leaver, 1978). However, Dickson *et al.* (1970), Collis (1976), and Kabuga *et al.* (1991) found that dominance was poorly correlated with live-weight. In other studies, both age and weight were correlated with dominance (Schein and Fohrman, 1955, Kabuga, 1992). It is worth bearing in mind that while each pair were within seven days in age and ten kilograms in weight of each other the variation within the other animals in the group may have been greater than this. Work looking at all the occupants of the pen would be needed to fully assess the role of dominance in the association between live-weight and milk feeding variables seen in Chapters 2 and 3. When predicting disease, an increase in live-weight has been shown to reduce the risk of developing disease (Chisholm *et al.*, 2021), however, no models including age were constructed. The size of this dataset meant that it was not prudent to test interactions between weight and disease status. Interestingly, a recent study showed that social dominance was reduced in sick calves (Vázquez-Diosdado *et al.*, 2025). Exploring the relationship between weight, disease status and social dominance would be an interesting area of further study.

Male calves tended to drink less milk at each visit in this study. However, sex was not in the final model for mean milk per visit in Chapter 2 (Riley *et al.*, 2023). There is some existing evidence that the behaviour of male calves differs to that of their female counterparts. Mahendran *et al.* (2023) found that male calves had fewer rewarded visits to the feeder than female calves. Previous research showed that male calves took less training to learn to use an automatic feeder (Wilson *et al.*, 2018).

This study aimed to control for the factors weight, age, social group, and sex in its design. However, this led to a very small dataset. From a total of one hundred and fourteen calves from which data was collected, only thirty-six calves were included in the milk feeding analysis and twenty-eight in the activity variable analysis. As shown in Table 3.3 and Table A1.1 neither of these data sets were balanced for sex

and the breed makeup of the two sexes was different, this limits the conclusions that can be drawn here. The unbalanced nature is not overly surprising as the larger data set that both datasets are derived from is also unbalanced (Table 3.1). Unfortunately, this was unavoidable as breeding decisions were made by the farm for production reasons rather than to meet the demands of upcoming trials. Furthermore, the breeding decisions for herd A were made according to a long-term study and all Holstein females born to dams from that herd were ineligible for the current study as they will be enrolled on to the long-term study later in life. The latest GB figures show that the number of beef-sired calves in the dairy-herd increased by 77% between 2013 and 2023 (Ruminant Health & Welfare Group, 2025). Only one paper to date has examined the association between behaviour and disease in dairy-beef calves (Mahendran *et al.*, 2023). While some research exists on male dairy calves e.g. Duthie *et al.* (2021) the majority is on female calves. Thus, there are many artificially reared calves that have minimal research on their behavioural response to disease. While in this study there was only associations between BRD and the milk visit length and milk consumed at each visit further research is warranted due to the limitations discussed above.

3.6 Conclusion

In conclusion, calves with BRD visited the milk for longer after disease and drank more milk before they became diseased. Calves on a lower milk allowance appear to show fewer behavioural changes with disease than their counterparts that are offered more milk. However, there are also differences in the sample sizes and methodologies between studies, thus making comparing results challenging. The association between BRD and calf behaviour warrants further exploration particularly in male and beef cross calves due to the limitations of this study and their increasing importance to the industry. Live-weight also has an association with milk feeding behaviours and will need to be considered in disease prediction models.

3.7 Bibliography

- BALLOU, M. A. 2012. Immune responses of Holstein and Jersey calves during the preweaning and immediate postweaned periods when fed varying planes of milk replacer. *Journal of Dairy Science*, 95, 7319-30.
- BARUCH, J., CERNICCHIARO, N., CULL, C. A., LECHTENBERG, K. F., NICKELL, J. S. & RENTER, D. G. 2019. Performance of multiple diagnostic methods in assessing the progression of bovine respiratory disease in calves challenged with infectious bovine rhinotracheitis virus and Mannheimia haemolytica. *Journal of Animal Science*, 97, 2357-2367.
- BATES, D., MÄCHLER, M., BOLKER, B. & WALKER, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67, 1-48.
- BAXTER-SMITH, K., MORE, J. & HYDE, R. 2022. Use of thoracic ultrasound on Scottish dairy cattle farms to support the diagnosis and treatment of bovine respiratory disease in calves. *Veterinary Record*, 190, e939.
- BINVERSIE, E. S., RUEGG, P. L., COMBS, D. K. & OLLIVETT, T. L. 2020. Randomized clinical trial to assess the effect of antibiotic therapy on health and growth of preweaned dairy calves diagnosed with respiratory disease using respiratory scoring and lung ultrasound. *Journal of Dairy Science*, 103, 11723-11735.
- BOLKER, B. 2021. *GLMM FAQ* [Online]. <https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#overdispersion>: Ben Bolker. Available: <https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#overdispersion> [Accessed 21/12/2021 2021].
- BORCHERS, M. R., CHANG, Y. M., TSAI, I. C., WADSWORTH, B. A. & BEWLEY, J. M. 2016. A validation of technologies monitoring dairy cow feeding, ruminating, and lying behaviors. *Journal of Dairy Science*, 99, 7458-7466.
- BORDERAS, T. F., RUSHEN, J., VON KEYSERLINGK, M. A. & DE PASSILLE, A. M. 2009. Automated measurement of changes in feeding behavior of milk-fed calves associated with illness. *Journal of Dairy Science*, 92, 4549-54.
- BOWEN, J. M., HASKELL, M. J., MILLER, G. A., MASON, C. S., BELL, D. J. & DUTHIE, C. A. 2021. Early prediction of respiratory disease in preweaning dairy calves using feeding and activity behaviors. *Journal of Dairy Science*, 104, 12009-12018.
- BROOM, D. M. & LEAVER, J. D. 1978. Effects of group-rearing or partial isolation on later social behaviour of calves. *Animal Behaviour*, 26, 1255-1263.
- BUSHBY, E. V., THOMAS, M., VÁZQUEZ-DIOSDADO, J. A., OCCHIUTO, F. & KALER, J. 2024. Early detection of bovine respiratory disease in pre-weaned dairy calves using sensor based feeding, movement, and social behavioural data. *Scientific Reports*, 14, 9737.

- CANTOR, M. C. & COSTA, J. H. C. 2022. Daily behavioral measures recorded by precision technology devices may indicate bovine respiratory disease status in preweaned dairy calves. *Journal of Dairy Science*, 105, 6070-6082.
- CANTOR, M. C., RENAUD, D. L., NEAVE, H. W. & COSTA, J. H. C. 2022. Feeding behavior and activity levels are associated with recovery status in dairy calves treated with antimicrobials for Bovine Respiratory Disease. *Scientific Reports*, 12, 4854.
- CHISHOLM, E., SCOLEY, G. & LAMROCK, F. 2021. 106. Using a multi-state modelling (MSM) approach to predict dairy calf health status using on-farm precision technology data. *Animal - Science Proceedings*, 12, 85.
- CLOSS, G. & DECHOW, C. 2017. The effect of calf-hood pneumonia on heifer survival and subsequent performance. *Livestock Science*, 205, 5-9.
- COLLIS, K. A. 1976. An investigation of factors related to the dominance order of a herd of dairy cows of similar age and breed. *Applied Animal Ethology*, 2, 167-173.
- CONBOY, M. H., WINDER, C. B., MEDRANO-GALARZA, C., LEBLANC, S. J., HALEY, D. B., COSTA, J. H. C., STEELE, M. A. & RENAUD, D. L. 2021. Associations between feeding behaviors collected from an automated milk feeder and disease in group-housed dairy calves in Ontario: A cross-sectional study. *Journal of Dairy Science*, 104, 10183-10193.
- CRAMER, C., PROUDFOOT, K. & OLLIVETT, T. 2020. Automated Feeding Behaviors Associated with Subclinical Respiratory Disease in Preweaned Dairy Calves. *Animals*, 10, 1-9.
- DICKSON, D. P., BARR, G. R., JOHNSON, L. P. & WIECKERT, D. A. 1970. Social Dominance and Temperament of Holstein Cows. *Journal of Dairy Science*, 53, 904-907.
- DUTHIE, C. A., BOWEN, J. M., BELL, D. J., MILLER, G. A., MASON, C. & HASKELL, M. J. 2021. Feeding behaviour and activity as early indicators of disease in pre-weaned dairy calves. *Animal*, 15, 100150.
- EFSA PANEL ON ANIMAL HEALTH AND ANIMAL WELFARE, NIELSEN, S. S., ALVAREZ, J., BICOUT, D. J., CALISTRI, P., CANALI, E., DREWE, J. A., GARIN-BASTUJI, B., GONZALES ROJAS, J. L., GORTAZAR SCHMIDT, C., HERSKIN, M., MICHEL, V., MIRANDA CHUECA, M. A., PADALINO, B., PASQUALI, P., ROBERTS, H. C., SPOOLDER, H., STAHL, K., VELARDE, A., VILTROP, A., JENSEN, M. B., WAIBLINGER, S., CANDIANI, D., LIMA, E., MOSBACH-SCHULZ, O., VAN DER STEDE, Y., VITALI, M. & WINCKLER, C. 2023. Welfare of calves. *EFSA Journal*, 21, e07896.
- FOOTE, NONNECKE, WATERS, PALMER, BEITZ, FOWLER, MILLER, JOHNSON & PERRY 2005a. Effects of Increased Dietary Protein and Energy on Composition and

Functional Capacities of Blood Mononuclear Cells from Vaccinated, Neonatal Calves. *International Journal for Vitamin and Nutrition Research*, 75, 357-368.

FOOTE, M. R., NONNECKE, B. J., FOWLER, M. A., MILLER, B. L., BEITZ, D. C. & WATERS, W. R. 2005b. Effects of Age and Nutrition on Expression of CD25, CD44, and Selectin (CD62L) on T-cells from Neonatal Calves. *Journal of Dairy Science*, 88, 2718-2729.

HARTIG, F. 2022. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. <https://CRAN.R-project.org/package=DHARMA>.

HIXSON, C. L., KRAWCZEL, P. D., CALDWELL, J. M. & MILLER-CUSHON, E. K. 2018. Behavioral changes in group-housed dairy calves infected with *Mannheimia haemolytica*. *Journal of Dairy Science*, 101, 10351-10360.

HOSSEINKHANI, A., DEVRIES, T. J., PROUDFOOT, K. L., VALIZADEH, R., VEIRA, D. M. & VON KEYSERLINGK, M. A. G. 2008. The Effects of Feed Bunk Competition on the Feed Sorting Behavior of Close-Up Dry Cows. *Journal of Dairy Science*, 91, 1115-1121.

JOHNSTON, D., KENNY, D. A., MCGEE, M., WATERS, S. M., KELLY, A. K. & EARLEY, B. 2016. Electronic feeding behavioural data as indicators of health status in dairy calves. *Irish Journal of Agricultural and Food Research*, 55, 159-168.

KABUGA, J. D. 1992. Social relationships in N'dama cattle during supplementary feeding. *Applied Animal Behaviour Science*, 34, 285-290.

KABUGA, J. D., GARI-KWAKU, J. & ANNOR, S. Y. 1991. Social status and its relationships to maintenance behaviour in a herd of N'dama and West African Shorthorn cattle. *Applied Animal Behaviour Science*, 31, 169-181.

KNAUER, W. A., GODDEN, S. M., DIETRICH, A., HAWKINS, D. M. & JAMES, R. E. 2018. Evaluation of applying statistical process control techniques to daily average feeding behaviors to detect disease in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, 101, 8135-8145.

KNAUER, W. A., GODDEN, S. M., DIETRICH, A. & JAMES, R. E. 2017. The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, 100, 5642-5652.

KUZNETSOVA, A., BROCKHOFF, P. B. & CHRISTENSEN, R. H. B. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1-26.

LENTH, R. V. 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means. <https://CRAN.R-project.org/package=emmeans>.

LOWE, G. L., SUTHERLAND, M. A., WAAS, J. R., COX, N. R., SCHAEFER, A. L. & STEWART, M. 2021. Effect of milk allowance on the suitability of automated

behavioural and physiological measures as early disease indicators in calves. *Applied Animal Behaviour Science*, 234.

MAHENDRAN, S. A., WATHES, D. C., BLACKIE, N. & BOOTH, R. E. 2023. Calf Health, Feeding and Social Behaviours within Groups Fed on Automatic Milk Feeders. *Ruminants* [Online], 3.

MAZEROLLE, M. 2020. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). <https://cran.r-project.org/package=AICcmodavg>.

MCCONNELL, C. S., NELSON, D. D., BURBICK, C. R., BUHRIG, S. M., WILSON, E. A., KLATT, C. T. & MOORE, D. A. 2019. Clarifying dairy calf mortality phenotypes through postmortem analysis. *Journal of Dairy Science*, 102, 4415-4426.

MCGUIRK, S. M. 2015. Calf Health Scoring Chart. https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf: School of Veterinary Medicine, Wisconsin-Madison University.

MELLOR, D. J. & STAFFORD, K. J. 2004. Animal welfare implications of neonatal mortality and morbidity in farm animals. *The Veterinary Journal*, 168, 118-133.

MONTES, M. E., DOUCETTE, J., BRITO, L. F. & BOERMAN, J. P. 2023. Environmental and biological factors that influence feeding behavior of Holstein calves in automated milk feeding systems. *JDS Communications*, 4, 379-384.

OLSON, A., SISCHO, W. M., BERGE, A. C. B., ADAMS-PROGAR, A. & MOORE, D. A. 2019. A retrospective cohort study comparing dairy calf treatment decisions by farm personnel with veterinary observations of clinical signs. *Journal of Dairy Science*, 102, 6391-6403.

PERTTU, R. K., PEITER, M., BRESOLIN, T., DÓREA, J. R. R. & ENDRES, M. I. 2023. Feeding behaviors collected from automated milk feeders were associated with disease in group-housed dairy calves in the Upper Midwest United States. *Journal of Dairy Science*, 106, 1206-1217.

R CORE TEAM 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

REVELLE, W. 2021. psych: Procedures for Psychological, Psychometric, and Personality Research. <https://CRAN.R-project.org/package=psych>. Northwestern University.

RILEY, B. B., DUTHIE, C.-A., CORBISHLEY, A., MASON, C., BOWEN, J. M., BELL, D. J. & HASKELL, M. J. 2023. Intrinsic calf factors associated with the behavior of healthy pre-weaned group-housed dairy-bred calves. *Frontiers in Veterinary Science*, 10, 1204580.

RUMINANT HEALTH & WELFARE GROUP 2025. GB Dairy Calf Strategy: A review of progress 2020-2023. <https://ruminanthw.org.uk/wp-content/uploads/2025/01/GB-dairy-calf-strategy-review-of-progress-Jan-2025-APP-compressed.pdf>.

SCHEIN, M. W. & FOHRMAN, M. H. 1955. Social dominance relationships in a herd of dairy cattle. *The British Journal of Animal Behaviour*, 3, 45-55.

SINNOTT, A. M., KENNEDY, E. & BOKKERS, E. A. M. 2021. The effects of manual and automated milk feeding methods on group-housed calf health, behaviour, growth and labour. *Livestock Science*, 244.

STEPHENS, D. B. 1974. Studies on the effect of social environment on the behaviour and growth rates of artificially-reared British Friesian male calves. *Animal Science*, 18, 23-34.

SWARTZ, T. H., FINDLAY, A. N. & PETERSSON-WOLFE, C. S. 2017. Short communication: Automated detection of behavioral changes from respiratory disease in pre-weaned calves. *Journal of Dairy Science*, 100, 9273-9278.

VÁZQUEZ-DIOSDADO, J. A., OCCHIUTO, F., SMITH, A.-L., THOMAS, M., GLOVER, A. & KALER, J. 2025. Social dominance in calves: Automatic detection of displacements at the feeder and its associations with age, health, and feeding behaviors. *Journal of Dairy Science*, 108, 10125-10135.

WICKHAM, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*, Cham, Springer-Verlag New York.

WICKHAM, H., AVERICK, M., BRYAN, J., CHANG, W., MCGOWAN, L., FRANÇOIS, R., GROLEMUND, G., HAYES, A., HENRY, L., HESTER, J., KUHN, M., PEDERSEN, T., MILLER, E., BACHE, S., MÜLLER, K., OOMS, J., ROBINSON, D., SEIDEL, D., SPINU, V., TAKAHASHI, K., VAUGHAN, D., WILKE, C., WOO, K. & YUTANI, H. 2019. Welcome to the Tidyverse. *Journal of Open Source Software*, 4, 1686.

WILSON, T. R., LEBLANC, S. J., DEVRIES, T. J. & HALEY, D. B. 2018. Effect of stall design on dairy calf transition to voluntary feeding on an automatic milk feeder after introduction to group housing. *Journal of Dairy Science*, 101, 5307-5316.

WORKMAN, A. M., KUEHN, L. A., MCDANELD, T. G., CLAWSON, M. L. & LOY, J. D. 2019. Longitudinal study of humoral immunity to bovine coronavirus, virus shedding, and treatment for bovine respiratory disease in pre-weaned beef calves. *BMC Veterinary Research*, 15, 161.

Chapter 4 Salivary pH, but not conductivity, is an indicator of diarrhoea in neonatal calves

4.0 Preface

Thus far, the behaviour of healthy calves and calves with bovine respiratory disease has been explored. Another key disease in group-housed dairy-bred calves is neonatal calf diarrhoea (NCD). In the UK, incidence of NCD may be as high as 65 per 100 calves in the first nine weeks of life (Johnson *et al.*, 2019). Calves with NCD may develop dehydration (Trefz *et al.*, 2013) and/or metabolic acidosis (Bednarski and Kupczyński, 2015). In addition to the behavioural changes seen in disease, there are a range of physiological changes. Saliva may reflect changes occurring in the blood serum and can be sampled non-invasively. It may therefore be possible to measure basic properties of saliva that correspond with disease. There is inconclusive evidence of the value of saliva osmolarity to detect dehydration in humans (Munoz *et al.*, 2013, Taylor *et al.*, 2012). Serum osmolarity and saliva conductivity are correlated (Lu *et al.*, 2019). Saliva anion gap has been shown to be correlated to the plasma anion gap (Hamada *et al.*, 2013), and saliva pH has been shown to be an indicator of metabolic syndrome in humans (Suzuki *et al.*, 2020). Thus, saliva pH may be useful in the detection of metabolic acidosis in calves. Unlike osmolarity and anion gap both pH and conductivity can be measured from small volumes using bench top metres.

This chapter had two objectives. Firstly, to understand the association between neonatal calf diarrhoea and saliva conductivity and pH in group housed artificially reared pre-weaned limit fed dairy-bred calves. And secondly, to understand the association between saliva conductivity and pH and physiological indicators of dehydration or acidosis in blood from group housed artificially reared pre-weaned limit fed dairy-bred calves.

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The permission of all co-authors was sought for inclusion in my thesis. The study concept and study design were carried out by myself with assistance from my supervisors. Most of the data collection was carried out by me. I curated the data, performed the data analysis, produced the original draft of the paper, and carried out most of the edits.

The supplementary material for this chapter can be found in Appendix 2.



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Salivary pH, but not conductivity, is an indicator of diarrhea in neonatal calves

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Neonatal calf diarrhea is a frequent disease of calves and may result in dehydration and metabolic acidosis. The disease causes mortality and reduces growth and future productivity. Early identification of disease improves calf outcomes and thus there is increasing interest in technological methods for detecting disease. Dehydration leads to the blood becoming more concentrated and this can be measured using serum osmolality. Research in humans has shown that saliva conductivity is correlated with serum osmolality. Saliva conductivity may therefore offer a non-invasive opportunity to assess hydration status in calves. Furthermore, as blood pH is a prognostic indicator and there is ion exchange in the salivary ducts, saliva pH may act as an indicator of metabolic acidosis. This observational study aimed to assess the relationship of saliva conductivity and pH with the clinical and biochemical parameters of calves suffering from neonatal calf diarrhea. One hundred and forty-one dairy-bred calves were recruited onto the study at approximately 1 week of age. The health of the calves was assessed daily. Calves had blood and saliva samples taken weekly until 25 days of age or the development of neonatal calf diarrhea. When calves developed diarrhea, they were sampled for three consecutive days. Hematocrit, plasma total protein, saliva pH and saliva conductivity were measured at each sampling. Saliva pH and saliva conductivity were measured using portable meters (*LAQUAtwin-pH-33* and *LAQUAtwin-EC22*). In a subset of 30 matched samples, serum proteins and electrolytes were also measured. Saliva conductivity was not associated with diarrhea or dehydration. Saliva pH was lower in calves with diarrhea, regardless of hydration status. The Lin's concordance correlation coefficients between saliva variables and hematocrit and strong ion difference were negligible. Dehydrated calves with diarrhea had a higher hematocrit and albumin and the lowest sodium and SID. Calves with diarrhea and no dehydration had a lower plasma total protein. While saliva conductivity has been associated with measures of dehydration in humans, this does not appear to be the case in calves. Saliva pH has not previously been considered for disease detection; however as it is associated with diarrhea, further research is warranted.

KEYWORDS

biomarker, conductivity, dehydration, hematocrit, neonatal calf diarrhea, pH, saliva, total protein

1 Introduction

Diarrhea is considered by both veterinarians and farmers to be one of the main threats to calf health (1). The prevalence and mortality rates for neonatal calf diarrhea (NCD) are variable in the literature. A UK study of 11 farms found that NCD affects an average of 48.2 per 100 calves in the first 10 weeks of life (2). However, a study of 10 dairy farms in the US found an incidence of 77 per 100 calves in the first 30 days of life, with 1.4% of diarrhea cases dying (3). A larger study in the US found that 17.2% of calves developed NCD in the pre-weaning period, with 8.5% of cases experiencing mortality (4). However, where NCD is recorded by researchers and not farm staff, the disease rates recorded are far higher. A study in the UK recorded an incidence of 64.5 per 100 calves in the first 8 weeks of life (5), a US study recorded a incidence of 85 per 100 calves under 28 days of age across four farms (6) and a Canadian study found an incidence of 97 per 100 calves under 49 days of age on a single farm (7). Together these data suggests that NCD is a widespread problem.

The NCD can cause impaired welfare (8), and has far reaching consequences in calves that have been affected. The number of weeks a dairy calf has diarrhea prior to weaning has been found to be negatively associated with average daily liveweight gain between 1 and 63 days of age (9). Holstein heifer calves which suffered from diarrhea pre-weaning had a lower average daily liveweight gain at weaning, were older at first calving and had a lower 305 day mature equivalent milk yield (10). These calves are also more likely to be removed from the herd in their first 300 days in milk in one study (11) but not in another (12). Beef calves suffering from severe diarrhea in the first 16 days of life were found to gain 34 kg less in the first 6 months of life (13). Furthermore, diarrhea on arrival to a veal unit has been associated with increased risk of disease and mortality during their time at the unit (14) but not with weight gain while at the unit (15). The proportion of days with diarrhea at a veal unit has been associated with a reduction in weight gain (16). A consequence of NCD can be dehydration, which in its severe form increases the risk of mortality (17).

Early detection of NCD and dehydration can lead to improved calf outcomes and reduce the costs associated with treatment, mortality, and reduced growth rates (18). This had led to an interest in technological solutions for disease detection. Existing research includes investigation of ear mounted accelerometers (18, 19), leg mounted accelerometers (20), automatic milk feeders (19, 21, 22) and infrared thermography (23). However, as yet, there is no research on the use of technologies to detect dehydration. Traditionally, dehydration is evaluated by using blood samples to measure the hematocrit (the percentage by volume of red blood cells in blood) and plasma total protein (PTP) in the laboratory. This is both invasive for the calf and means that there is a time lag before results are available to the farmer or veterinary surgeon. There is therefore an opportunity to explore other parameters that can be measured in a non-invasive manner. This study aimed to look at measurements from saliva that could be measured using a portable meter.

Saliva osmolality has been shown to increase with dehydration in humans (24). One study has demonstrated that saliva osmolality increases linearly with body mass loss (a measure of dehydration) in dehydrated humans (25), although another study suggested that this relationship was non-linear (26). The diagnostic accuracy (receiver-operating characteristic-area under the curve) of saliva osmolality for

mild intracellular dehydration in humans was 0.70 (confidence interval 0.51–0.85) (27). Saliva conductivity is correlated with serum osmolality in humans (28) and thus has potential to be used to monitor dehydration, especially as conductivity can be measured with a portable meter. Previous research into calf saliva has included measuring saliva cortisol for stress (29), and saliva immunoglobulins to monitor for failure of passive transfer (30). However, spectrophotometric methods such as enzyme-linked immunosorbent assays are required to measure all these analytes, and this requires a diagnostic laboratory.

The NCD also frequently results in metabolic acidosis (31). It is thought that this is due to the production of D-lactate in the colon (31), and sodium loss through the gastrointestinal tract (32). In young calves, metabolic acidosis is associated with an increased risk of mortality (33). Blood gas analysis has been considered beneficial for assessment of diarrhea in calves (32) as it gives a more detailed assessment of the nature of the electrolyte imbalances present (34). However, the equipment required is costly (34). It has been previously suggested that the pH of whole blood could be an alternative to blood gas analysis, due to the high correlation between blood pH and a clinical assessment scoring system (34). Blood pH has also been shown to be a predictor for mortality (17). However, a test that can be done pen-side would allow more immediate treatment, while minimizing invasive procedures on sick calves will reduce stress. Furthermore, there is potential for the development of systems to collect calf saliva to examine the health status of a group as has previously been suggested in pigs (35) or possibly an individual if measurement at the teat is possible. If individual identification of dehydrated calves is possible at an early stage this would allow earlier intervention and thus improve welfare. Saliva pH has previously been measured in calves when examining the development of the rumen (36). Thus, saliva pH could potentially be a tool for the detection of metabolic acidosis in calves.

This observational study aimed to assess the relationship of saliva conductivity and pH with the clinical and biochemical parameters of calves suffering from NCD.

2 Materials and methods

2.1 Animal management

The animal work for this study was conducted at the SRUC Dairy Research and Innovation Centre, Crichton Royal Farm, Dumfries, UK under the approval of the Animal Experiment Committee of SRUC (DAI AE 06–2022). One hundred and forty-one dairy-bred calves were recruited from the all year round calving Holstein herd, and managed under the normal rearing conditions of the farm. This sample size was based on the previous understanding of NCD prevalence on the farm. Each calf received 4 L of defrosted cow's colostrum by stomach tube within 8 h of birth. Calves were housed in straw or woodchip bedded individual hutches from birth to approximately 7 days of age, where they were fed six liters of milk replacer split between two meals daily (*Maximum +, Carrs Billington Agriculture (Sales) Ltd., Carlisle*, crude protein 24%, crude oils and fats 20%, crude ash 7.5%, calcium 0.9%, sodium 0.5%, phosphorus 0.7%, 176 g/L). Calves had *ad-libitum* access to concentrate pellets (*Ambition calf and Omnigen nuts, Mole Valley Feed Solutions, South Molton*, dry

matter 86.2%, crude protein 18%, crude oils and fats 4.6%, crude fiber 9.2%, crude ash 9.2%, Sodium 0.5%. Selenium 0.4 mg/kg, copper 19 mg/kg, Vitamin E 60 mg/kg) and water. Due to a history of severe acute cryptosporidiosis in calves less than 7 days of age, calves received paromomycin sulfate in milk for their time in the individual hutches (*Parofoor, Huvepharma NV, Antwerp, Belgium*, 10 g/calf/day) as advised by the farm's independent veterinary surgeons.

Calves were transferred to group pens containing 10–12 animals at approximately 7 days of age. Group pens were straw-bedded and consisted of an igloo (3.9 m × 4.4 m, 2.2 m high) and an adjacent covered pen (5.1 m × 5.1 m) (stocking density 3.6–4.3 m²/calf). Each calf had access to seven liters of milk replacer daily (as above) using an automatic milk feeder (*custom built for this calf unit, BioControl Norway As, Grimstad Gård, Norway*). Calves had *ad libitum* access to water, concentrate (as above) and straw.

2.2 Health assessment

Calves were recruited to the study 24 h after entry into the group pen. Health scoring was carried out daily, including the Wisconsin calf health score (HEALTH) (37), a long-established tool that includes temperature, ocular and nasal discharge, ear posture, and cough. In addition, tail, perineum and hind leg cleanliness (CLEAN) and feces were scored on a scale of 0–3. The scoring system for both CLEAN and the feces score are shown in Table 1. Feces was scored whenever defecation was observed while in the pen for scoring. Skin tent elasticity and capillary refill time were recorded to determine whether the calf was suffering from dehydration. Skin tent elasticity was measured behind the shoulder. A calf was designated as being dehydrated when the rebound of the skin tent took more than 3 s. Capillary refill time was measured using the oral mucosa. These measures were chosen as they were easy to train and were both associated with a >4% reduction in hydration in young calves when over 3 s (38). Additional criteria were needed as HEALTH does not consider dehydration and in group housed calves feces can only be linked to an individual if defecation is observed. NCD was classified as a feces score of ≥2 or a CLEAN score of ≥2. Scoring was predominantly carried out by one person (BR, 85%) with the remainder being carried out by three research technicians. All four scorers trained together and compared until consistent.

Disease was not induced in this study. Where the research team detected spontaneously occurring disease, it was reported to the farm

TABLE 1 The criteria for the tail, perineum and hind leg cleanliness (CLEAN) and feces scores.

Score	Scoring criteria	
	CLEAN	Feces (38)
0	Clean calf or with a small amount of dried feces on tail/perineum/hind legs	Formed feces
1	A large amount of dried feces or some pasty feces on tail/perineum/hind legs	Pasty feces
2	Wet feces on tail/perineum/hind legs	Loose feces that did not sift through bedding
3	A very wet tail/perineum or a large amount of feces on tail/perineum/hind legs	Liquid feces that sifted through the bedding

manager based on the following pre-agreed severity criteria: low level of milk consumed in a calf with NCD (<2 L by 10:30 am), depressed calf demeanor, fever, Wisconsin score >2 or dehydration. Calves were treated according to protocols agreed with the veterinary surgeon responsible for the farm. This was a non-steroidal anti-inflammatory for all calves (meloxicam, *Meloxidyl 20 mg/mL solution for injection for cattle, pigs and horses, CEVA Animal Health Ltd., Woodburn Green, Buckinghamshire*, 0.5 mg/kg subcutaneously), a 3 day course of antibiotic for those with a fever or blood in the feces (trimethoprim and sulfadiazine, *Norodine 24 solution for injection, Norbrook, Newry, County Down*, 15 mg/kg intramuscularly) and oral rehydration for dehydrated/dull calves (*Life-Aid Xtra, Norodine 24 solution for injection, Norbrook, Newry, County Down*, mixed with 2 L water and administered by stomach tube).

2.3 Samples

Saliva and blood samples were taken from healthy calves on the day of recruitment and weekly thereafter (“non-diseased” samples). When NCD was detected from feces or CLEAN score, saliva and blood samples were taken on the day of detection and for two subsequent days (“diseased” samples). Fecal samples were also taken on the first day that NCD was detected. Calves finished the trial at 26 days of age or after one episode of NCD.

2.3.1 Feces

Each feces sample was tested using an immunochromatography tests for rotavirus, cryptosporidium, *E. coli* K99 and bovine coronavirus (*Expertis scour check test, MSD Animal Health, Walton, Milton Keynes* or *Surecheck 4, Nimrod Veterinary Products Ltd., Moreton-in-Marsh Gloucestershire*) according to the provided instructions.

2.3.2 Saliva

Saliva was sampled using a Salivette® sponge (*Salivette®, SARSTEDT AG & Co. KG, Sarstedtstraße, Nümbrecht, Germany*) held in a pair of forceps, which was placed in the calf's mouth for 1 min. This was based on experience during a pilot trial to allow sufficient volume to be collected. The sponge was then placed in the top portion of the Salivette tube. The forceps were disinfected between calves.

The Salivette tubes were centrifuged at 981 g for 15 min and the sponge and top portion of the tube removed. The saliva conductivity was measured by placing approximately one-sixth of the sample on the sensor of the conductivity meter (*LAQUAtwin-EC-22, HORIBA Advanced Techno Co. Ltd., Kisshoin Minami-ku, Kyoto, Japan*). Each sample was measured three times and the mean calculated. The saliva pH was measured in the same way using a pH meter (*LAQUAtwin-pH-33, HORIBA Advanced Techno Co. Ltd., Kisshoin Minami-ku, Kyoto, Japan*). Saliva was predominately analyzed within 8 h, if analysis within 30 h was not possible then saliva was frozen in aliquots at −20°C. There was no difference between the pH of fresh and defrosted saliva (results not shown).

2.3.3 Blood

Blood samples were taken from the jugular vein. The hair covering the jugular groove was clipped on entry to the study. Venipuncture was performed and 6 mL of blood was taken into a clot activator tube (*VACUETTE® 6 mL CAT Serum, Greiner Bio-One Ltd., Stonehouse,*

Gloucestershire) and a further 4 mL into a potassium Ethylenediaminetetraacetic (EDTA) tube (VACUETTE® 4 mL K2EDTA, Greiner Bio-One Ltd., Stonehouse, Gloucestershire). Serum was collected by centrifuging the clot activator tube at 981 g for 10 min and removing the serum using a Pasteur pipette. This was then frozen at -20°C for subsequent analysis.

Hematocrit was measured by centrifuging (3 min at 13,000 g) two capillary tubes of whole EDTA blood per sample. These were then measured using a hematocrit reader (39) and the mean calculated. PTP was measured by centrifuging whole EDTA blood (10 min at 981 g) and then pipetting the plasma on to a refractometer (RHC-200/ATC, Mag-Tek Dual Scale Refractometer, Gain Express Holdings Ltd., To Kwa Wan, Kowloon, Hong Kong). Each sample was measured twice and the mean calculated (39).

2.4 Data processing and analysis

All data were recorded in Microsoft Excel and data processing was carried out using the tidyverse package (40) in R (41) using the R studio graphical interface (*R studio, Boston, Massachusetts*). Any non-diseased samples taken within 2 days preceding the development of NCD or on a day where the HEALTH score was >4 (classified as intermediate or diseased) were excluded from all further analysis. The remaining non-diseased samples were classified as “healthy.” The diseased samples were sub-classified according to the hydration status of the calf on the day of sampling. Specifically, samples taken on a day where the skin tent elasticity was normal were classified as Neonatal Calf Diarrhea Hydrated (NCD-H) and samples taken on a day where the skin tent return was delayed were classified as Neonatal Calf Diarrhea Dehydrated (NCD-D).

To test the hypotheses that saliva conductivity, saliva pH, PTP and hematocrit were associated with NCD and dehydration, general linear mixed models were constructed for each of these parameters in turn. The calf identity nested within group was used as a random effect in all models. Disease status (Healthy, NCD-H or NCD-D), sex, sire breed-type, the interaction between sex and sire breed-type, age, the interaction between disease and age, the interaction between age and sex, age at inclusion into the group pen, and date were all included in the maximal model. The step() function [stats package, (41)] was used to carry out backwards model selection using the Akaike information criterion (AIC). The final model was checked using the simulateResidual() and plotQQunif() functions in the DHARMA package (42). Where appropriate, the response variable was transformed. A natural log transformation was used on the saliva conductivity and a square transformation was used on saliva pH. The model output was calculated using the summary() (43) and confint() functions (41) with the anova() function (41) used to calculate the numerator degrees of freedom (NDF) and denominator degrees of freedom (DDF). The estimated marginal means and pairwise comparisons were calculated from the model using the emmeans() function in the emmeans package (44), and plotted using ggplot2 (45).

In order to further explore the potential of saliva parameters to detect dehydration, correlations between hematocrit (a hematological proxy for dehydration) and saliva pH and conductivity were tested. Scatter diagrams were plotted using ggplot2 (45). Lin's correlation concordance coefficient (pC) were tested using the CCC() function in the DescTools package (46).

2.4.1 Serum biochemistry

To examine the relationship between saliva parameters, metabolic acidosis and serum proteins in calves with diarrhea, a subset of samples were selected. A balanced dataset was created by matching each calf with NCD-D with a healthy and NCD-H calf on the following basis: date of sampling (within 14 days), age (within 7 days), sire breed-type, sex, and disease stage. Each calf was used only once. The matching criteria were chosen based on the results of the healthy calf analysis (Supplementary material) and diseased models.

Sodium, potassium, chloride, serum total protein (STP) and albumin were measured in defrosted serum samples using an AU80 Chemistry Analyzer with ISE unit (Beckman Coulter Ireland Inc., Lismeehan, O'Callaghans Mills, Co. Clare, Ireland). Globulin was calculated by the analyzer from the albumin and STP. The strong ion difference (SID, a proxy for metabolic acidosis) was calculated as below (47).

$$\text{SID} = (\text{Sodium} + \text{Potassium}) - \text{Chloride}$$

IgG was measured using radial-immunodiffusion, following the manufacturer's instruction using kits provided by SCCL (SCCL, 30 Molaro Place, Saskatoon, SK, Canada, S7K 6A2).

Linear models were built for each of hematocrit, saliva conductivity, saliva pH, STP, albumin, globulin, potassium, sodium, chloride and SIG using the lm() function in the stats package (41). Disease status (Healthy, NCD-H or NCD-D), age, sex, sire breed-type, season, age at inclusion and the interaction between age and disease status were tested as fixed effects. The step procedure in the stats package (41) was used to perform backwards model selection. The selected model was then checked using the simulateResiduals() and plotqqunif() functions in the DHARMA package (42). The outputs of the models were calculated using the summary(), anova() and confint() functions (41). The estimated marginal means were calculated using the emmeans() function in the emmeans package (44) and plotted using ggplot2 (45).

In order to further explore the potential of saliva parameters to detect metabolic acidosis in calves, correlations between SID (a proxy for acidosis) and saliva pH and conductivity were explored. Scatter diagrams were plotted using ggplot2 (45). pC was tested using the CCC() function in the DescTools package (46).

3 Results

Of the 141 calves recruited onto the study, 108 developed NCD. Ninety-eight of these did not develop dehydration and 10 of these cases developed dehydration. Each dehydrated calf was only dehydrated for 1 day.

3.1 Pathogens

All 108 calves that developed NCD had a feces sample tested. Of these 49 were positive for Cryptosporidium, 27 were negative for all four pathogens tested, 14 were positive for Bovine Rotavirus,

five were positive for *Cryptosporidium*, Bovine Rotavirus and Bovine Coronavirus, four were positive for *Cryptosporidium* and Bovine Rotavirus, three were positive for *Cryptosporidium* and Bovine Coronavirus, two were positive for Bovine Coronavirus, one was positive for Bovine Rotavirus and Bovine Coronavirus and one was positive for *Cryptosporidium*, Bovine Rotavirus and *E. coli* K99.

3.2 The association of neonatal calf diarrhea and hydration status with blood and saliva variables

There were 488 sampling events from 139 calves available for analysis. One calf was excluded due to developing clostridial abomasitis the day after sampling and another had a HEALTH score >4 at each sampling event so was excluded from analysis. The number of samples in each disease category (Healthy, NCD-H, NCD-D) as well as the sex and sire breed-type of the corresponding calves are shown in [Supplementary Table S1](#). The descriptive statistics of the continuous calf variables, and the saliva and blood parameters from this data set are shown in [Supplementary Table S2](#).

3.2.1 Calves with diarrhea had a lower saliva pH

Saliva conductivity was not associated with disease status (when compared to healthy calves; NCD-H: $p = 0.12$; NCD-D: $p = 0.692$, [Table 2](#)). Calves with diarrhea had a lower saliva pH than their healthy counterparts (NCD-H: $p < 0.01$, and NCD-D: $p < 0.01$, [Figure 1](#)). Dehydrated calves were not different from NCD-H calves ($p = 0.07$, [Figure 1](#)). Calves that were introduced into the group pen at an older age had a higher saliva conductivity ($p = 0.02$, [Table 2](#)).

3.2.2 Hematocrit was highest in dehydrated calves and protein lowest in NCD-H calves

Hematocrit was highest in dehydrated calves (relative to healthy calves, $p < 0.01$, relative to NCD-H calves, $p < 0.01$, [Figure 2A](#)). There was no difference between healthy and NCD-H calves ($p = 0.90$, [Figure 2A](#)).

NCD-H calves had the lowest PTP when compared to healthy calves ($p < 0.01$, [Figure 2B](#)). NCD-D calves were not different from either healthy or NCD-H calves ($p = 0.81$, and $p = 0.07$ respectively, [Figure 2B](#)).

Male calves had higher hematocrit than female calves ($p = 0.03$, [Table 2](#)). Both hematocrit and PTP were lower in dairy-sired calves: than in their beef-sired counterparts ($p < 0.01$, and $p < 0.01$, respectively, [Table 2](#)). PTP was highest in younger calves and declined as calves got older ($p < 0.001$, [Table 2](#)).

TABLE 2 The association of neonatal calf diarrhea and hydration status with changes in saliva and blood parameters in artificially reared calves.

Variable	Factor	Level	Number of calf days	Estimate	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t-value	p-value
Saliva conductivity (mS/cm) ^a	Disease	Healthy	164 ^c	Reference	Reference	2	453.62	Reference	Reference
		NCD-H ^b	314 ^d	1.03	0.99 – 1.08			1.543	0.123
		NCD-D ^b	10 ^e	1.03	0.85 – 1.11			–0.397	0.692
	Age at inclusion into the group pen		488 ^f	1.06	1.01–1.10	1	138.15	2.384	0.019
Saliva pH ^h	Disease	Healthy	164 ^c	Reference	Reference	2	469.27	Reference	Reference
		NCD-H	314 ^d	–1.37	–1.38 – –0.68			–3.196	0.002
		NCD-D	10 ^e	–1.97	–2.51 – –1.20			–3.160	0.002
Hematocrit (%)	Disease	Healthy	164 ^c	Reference	Reference	2	399.10	Reference	Reference
		NCD-H	314 ^d	–0.13	–0.96 – 0.70			–0.307	0.759
		NCD-D	10 ^e	4.02	1.44–6.61			3.059	0.002
	Sex	Female	228 ^g	Reference	Reference	1	128.51	Reference	Reference
		Male	260 ^h	–1.84	–3.52 – –0.16			–2.168	0.032
	Sire breed-type	Beef	393 ⁱ	Reference	Reference	1	133.06	Reference	Reference
		Dairy	95 ^j	–3.15	–5.31 – –0.98			–2.893	0.005
Plasma total protein (g/dL)	Disease	Healthy	164 ^c	Reference	Reference	2	432.25	Reference	Reference
		NCD-H	314 ^d	–0.18	–0.28 – –0.08			–3.590	<0.001
		NCD-D	10 ^e	0.08	–0.16 – 0.32			0.620	0.536
	Sire breed-type	Beef	393 ⁱ	Reference	Reference	1	136.05	Reference	Reference
		Dairy	95 ^j	–0.33	–0.55 – –0.11			–3.011	0.003
	Age	488 ^f	–0.03	–0.04 – –0.02	1	472.28	–5.697	<0.001	

The results shown are from the final linear mixed models. Variables shown in bold have $p < 0.05$.

^aValues have been back transformed.

^bNCD-H: neonatal calf diarrhea-hydration status normal; NCD-D: neonatal calf diarrhea-dehydrated.

^c105 calves, ^d109 calves, ^e10 calves, ^f139 calves, ^g75 calves, ^h64 calves, ⁱ112 calves, ^j27 calves.

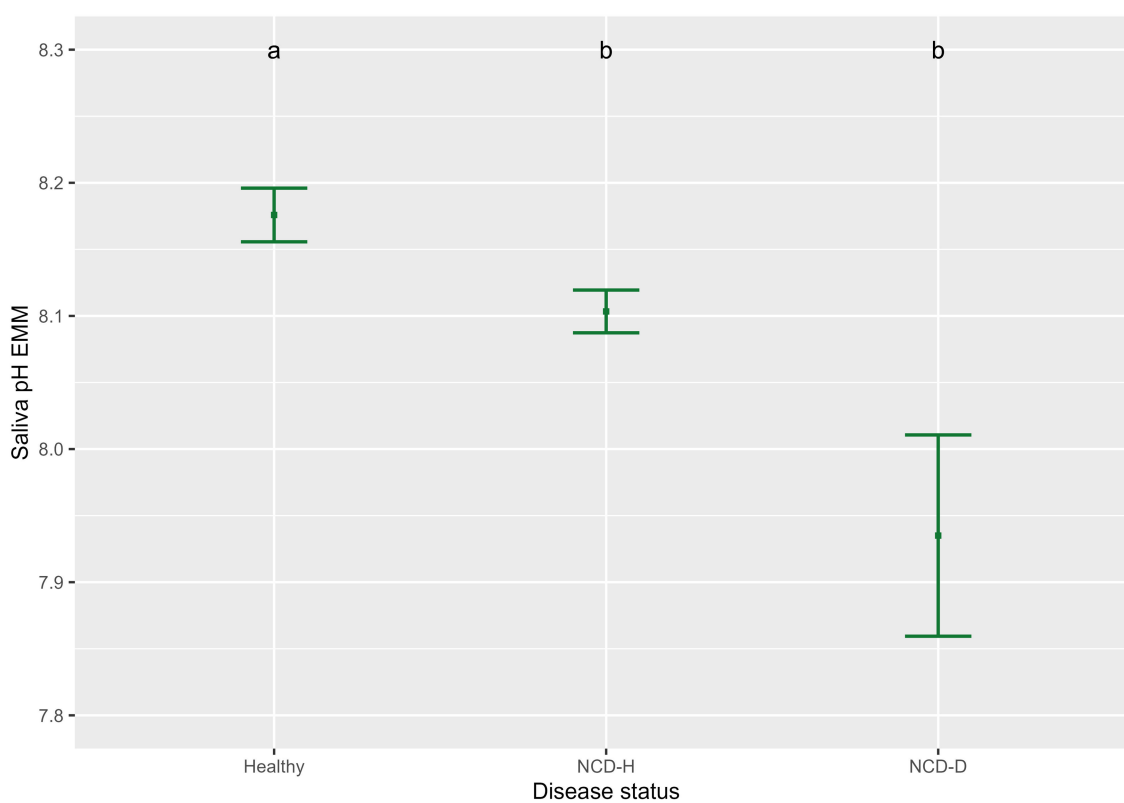


FIGURE 1

Association of neonatal calf diarrhea and hydration status with saliva pH. Plots and error bars denote the estimated marginal means (EMM) and corresponding standard errors calculated from the final model. Differing letters indicate statistically significant differences. Healthy: 164 calf days (105 calves), NCD-H: neonatal calf diarrhea-hydration status normal: 314 calf days (109 calves); NCD-D: neonatal calf diarrhea-dehydrated: 10 calf days (10 calves).

3.2.3 Saliva parameters were not correlated with hematocrit

Saliva parameters were explored for the Lin's correlation concordance with hematocrit, which is a proxy for dehydration. The correlation between both saliva pH and saliva conductivity and hematocrit was negligible ($pC = -0.00$, confidence interval = $-0.00 - -0.00$, and $pC = -0.00$, confidence interval = $-0.00 - 0.00$, respectively, [Supplementary Figure S1](#)).

3.3 The association of disease with serum biochemistry parameters

Thirty calf days were used to generate a balanced data set across the three disease status categories (i.e. 10 healthy days, 10 NCD-H days, and 10 NCD-D days). There were four sets of male beef calves, three sets of female beef calves and three sets of female dairy calves. The descriptive statistics of the continuous calf variables and the serum biochemistry parameters are shown in [Supplementary Tables S4, S5](#).

3.3.1 Serum proteins

There was no difference in STP between the three groups (compared to healthy calves: NCD-H: $p = 0.87$ and NCD-D: $p = 0.53$, [Table 3](#)).

Albumin was highest in NCD-D calves compared to their healthy or NCD-H counterparts ($p < 0.01$, and $p < 0.01$, respectively, [Figure 3B](#)). There was no difference between NCD-H and healthy calves ($p = 0.99$, [Figure 3A](#)).

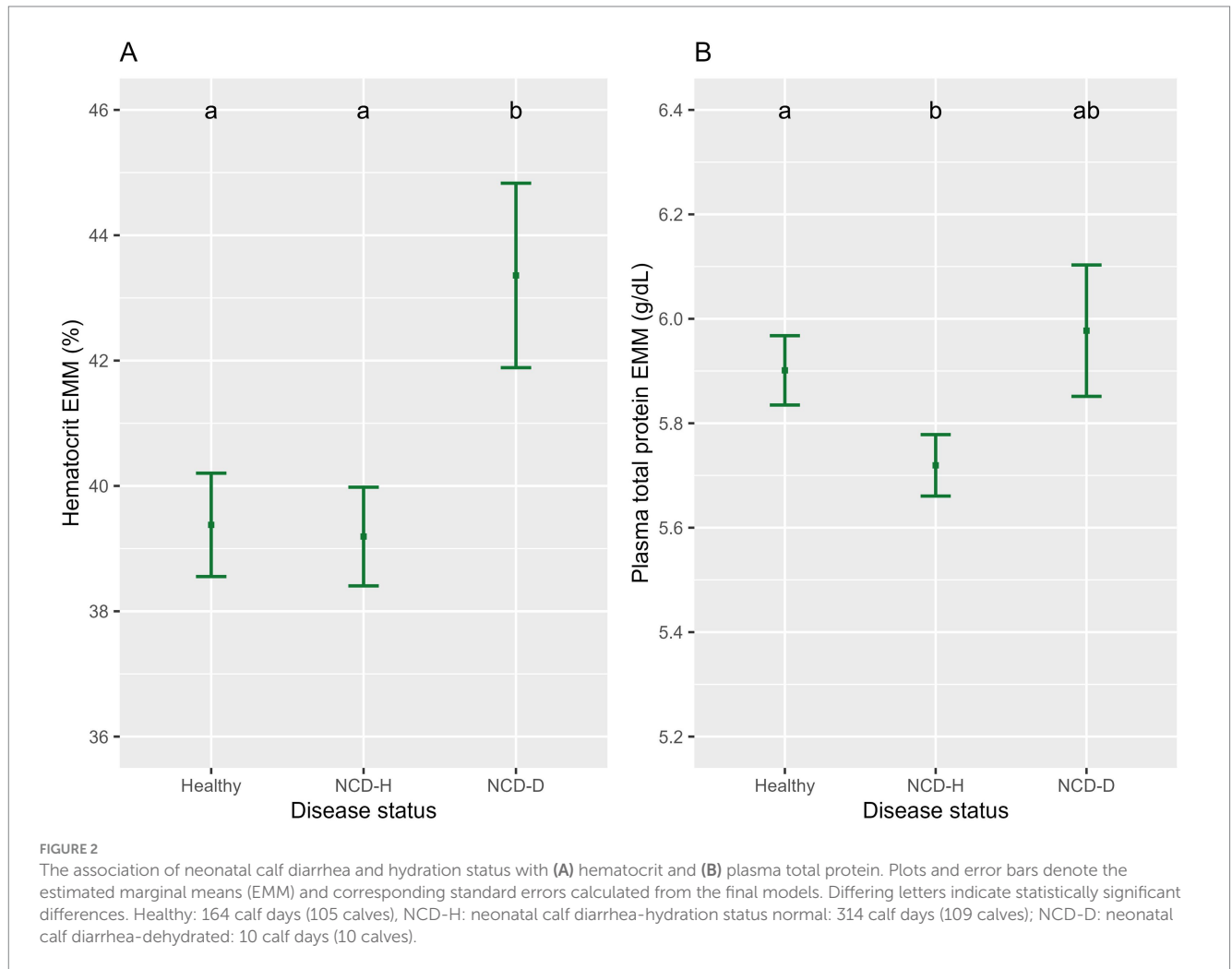
There was no difference in globulin concentrations between the three groups (compared to healthy calves: NCD-H: $p = 0.93$; NCD-D: $p = 0.20$, respectively, [Table 3](#)).

IgG was not different between the three groups (when compared to healthy calves, NCD-H; $p = 1.00$, NCD-D; $p = 0.17$, [Figure 3B](#)).

Older calves had a higher level of albumin than their younger counterparts ($p < 0.01$, $t = 2.986$, $DF = 2$, [Table 3](#)). Age was included in the final models for albumin, globulin and IgG as it improved model fit ($p = 0.08$, $p = 0.19$ and $p = 0.11$ respectively, [Table 3](#)). Sex and age at inclusion into the group pen were included in the final model for albumin as they improved the model fit ($p = 0.11$ and $p = 0.13$ respectively, [Table 3](#)).

3.3.2 Dehydrated calves had a lower strong ion difference

Calves with NCD-D had the lowest sodium concentration (compared to healthy calves: $p < 0.01$, compared to NCD-H calves: $p = 0.01$, [Figure 4A](#)). Sodium concentrations did not differ between NCD-H and healthy calves ($p = 0.73$, [Figure 4A](#)). Age and the interaction between age and disease were included in the final model



as they improved model fit ($p = 0.66$ and $p = 0.12$, respectively, Figure 4A).

Potassium was lowest in NCD-H calves (when compared to healthy calves: $p = 0.04$, Table 4), however this was no longer true in the pairwise comparison ($p = 0.73$, Figure 4B). There was no difference between NCD-D calves and healthy or NCD-H calves ($p = 0.78$ and $p = 0.34$ respectively, Figure 4B). Younger calves had the highest serum potassium ($p = 0.010$, Figure 4B). There interaction between age and disease was included as it improved model fit ($p = 0.08$, Figure 4B).

Chloride was lowest in NCD-H calves (when compared to healthy calves, $p = 0.03$, Table 4). Chloride was also lower in NCD-D calves compared to their healthy counterparts ($p = 0.04$, Table 4). However no pairwise comparisons of estimated marginal means were significant ($p > 0.05$). Age was not associated with serum chloride ($p = 0.52$, Figure 4C). However, there was an interaction between disease and age ($p = 0.04$, Figure 4C).

The SID was lowest in NCD-D calves (Healthy: $p < 0.001$, NCD-H: $p < 0.01$, Figure 4D). Healthy calves and NCD-H calves were not different from each other ($p = 0.98$, Figure 4D).

3.3.3 Saliva parameters were not associated with strong ion difference

To explore the association between saliva parameters and metabolic acidosis, the Lin's concordance correlations between saliva

parameters and SID were calculated. Neither saliva conductivity or saliva pH were associated with SID ($pC = -0.00$, confidence interval = $-0.00 - -0.01$, and $pC = -0.00$, confidence interval = $-0.00 - 0.00$, respectively, Supplementary Figure S2).

4 Discussion

This study aimed to assess the relationship of saliva conductivity and pH with the clinical and biochemical parameters of calves suffering from neonatal calf diarrhea (NCD). Saliva conductivity was not associated with NCD with or without dehydration. Saliva pH was associated with NCD with or without dehydration. The Lin's concordance correlation coefficient with hematocrit or strong ion difference (SID) was negligible, however. Dehydrated calves had a higher hematocrit than calves with NCD without dehydration or healthy calves. Calves with NCD and no dehydration had a lower PTP than healthy or dehydrated calves. Albumin was higher in dehydrated calves. There was no effect of disease with or without dehydration on STP, globulin or IgG. Calves with NCD and dehydration had the lowest sodium and SID.

The incidence of disease in this study was 77 per 100 calves under 25 days of age, this was higher than previously reported in the UK by Johnson et al. (2) (mean 48 per 100 calves in the first 10 weeks of life,

TABLE 3 The association of neonatal calf diarrhea and hydration status with serum protein parameters in artificially reared calves.

Variable	Factor	Level	Number of calves ^b	Estimate	Confidence interval	Degrees of freedom	t-value	P-value
Serum total protein (g/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H ^a	10	-0.54	-7.01 – 5.93		-0.171	0.865
		NCD-D ^a	10	-2.02	-8.49 – 4.45		-0.641	0.527
Albumin (g/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H	10	-0.14	-2.00 – 1.71		-0.160	0.874
		NCD-D	10	3.35	1.47–5.23		3.683	0.001
	Sex	Female	18	Reference	Reference	1	Reference	Reference
		Male	12	1.48	-0.38 – 3.33		1.645	0.114
	Sire breed-type	Beef	21	Reference	Reference	1	Reference	Reference
		Dairy	9	1.76	-0.24 – 3.75		1.820	0.082
	Age			30	0.28	0.09–0.47	1	2.986
Age at inclusion into the group pen			30	1.05	-0.34 – 2.45	1	1.559	0.133
Globulin (g/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H	10	0.33	-6.95 – 7.61		0.094	0.926
		NCD-D	10	-4.58	-11.82 – 2.65		-1.302	0.204
	Age			30	-0.45	-1.14 – 0.24	1	-1.340
IgG (g/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H	10	0.49	-9.69 – 10.67		0.098	0.923
		NCD-D	10	-9.16	-19.28 – 0.96		-1.860	0.074
	Age			30	-0.78	-1.74 – 0.19	1	-1.653

The results shown are from the final linear models. Variables shown in bold have $p < 0.05$.

^aNCD-H: neonatal calf diarrhea-hydration status normal; NCD-D: neonatal calf diarrhea-dehydrated.

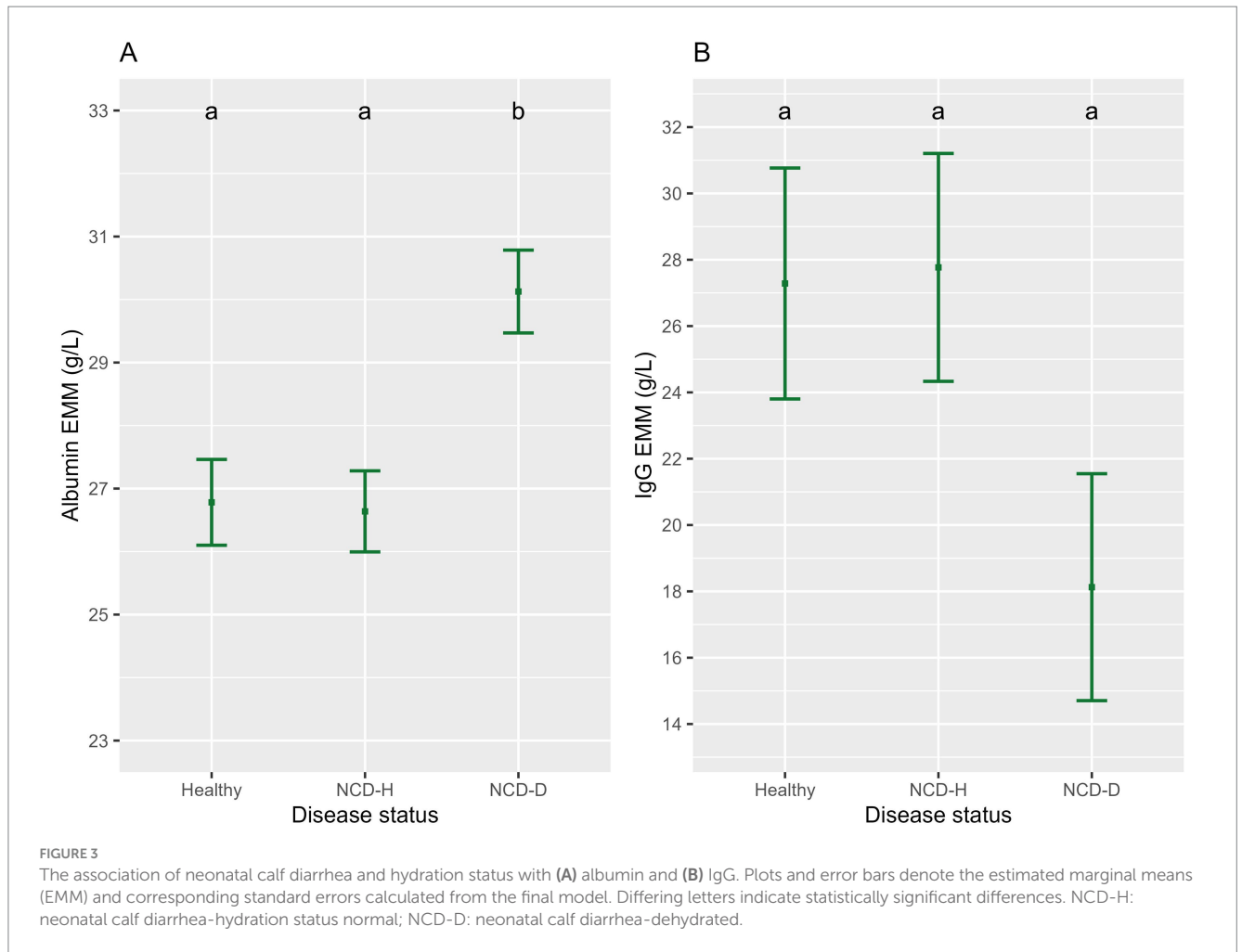
^bAs each calf was only included once in this dataset the number of calves and the number of calf days are equivalent.

range: 24–74) and Johnson et al. (5) (65 per 100 calves in the first 8 weeks of life), this may be due to the daily monitoring of the calves, whereas the previous studies monitored weekly. Previous studies that have carried out daily monitoring have recorded incidence rates of 85 per 100 calves under 28 days of age (6) and 97 per 100 calves under 49 days of age (7). While NCD is a major cause of mortality in calves (4) there were no cases of mortality due to NCD in this study. This is most likely due to early and aggressive oral rehydration therapy when dehydrated calves were identified. It is worth noting however, that the dehydrated calves were all identified by the research staff prior to them being identified by the farm staff and thus treatment was initiated earlier than it would have been in a normal farm situation.

Saliva conductivity was of interest as it has been previously shown to be correlated with serum osmolality in humans (28). The ionic concentration of the fluid affects the conductivity of the fluid in a non-linear function, the use of conductivity as a proxy for osmolality is incorporated into some urinalysis machines (48). Interestingly, previous work on saliva osmolality in humans showed an association between saliva osmolality and body water in humans who have exercised, but not in humans that had undergone passive dehydration (24). Ely et al. (49) found that saliva osmolality in humans was affected by a brief water rinse of the mouth, although the effect lasted less than 15 min. It was not possible to control whether calves had accessed the milk or water within 15 min prior to sampling and so this cannot be excluded as potentially having affected the results of this study. While this study did not identify an association between diarrhea with

or without dehydration and saliva conductivity, the limited number of dehydrated calves ($n = 10$) with each only scored as dehydrated for 1 day, means that we cannot conclusively exclude an association between saliva conductivity and moderate to severe dehydration in neonatal calves. In humans, saliva has a role in buffering the oral environment (50). However, in ruminants, far higher levels of sodium, bicarbonate and phosphate are found than in monogastric animals. Saliva is also continuously produced and is produced at high volumes, this is due to saliva having a key role in the buffering of the rumen (51). These differences in composition may explain why the results seen here were different to those seen in previous human studies.

Saliva pH was lower in calves with diarrhea. This is consistent with the presence of concurrent metabolic acidosis in calves with NCD. Metabolic acidosis is caused by an increase in D-lactate caused by fermentation of mal-absorbed carbohydrates in the colon, an increase in L-lactate due to dehydration and decreased tissue perfusion and the loss of bicarbonate in the feces (52). Sodium may also be lost in feces (32). A previous study found that the pH of whole blood was negatively correlated with disease severity (34). In this study there was no difference between dehydrated and normally hydrated calves with NCD, this may be due to the small number of dehydrated calves and warrants further investigation. However, Lorenz (53) previously found no correlation between D-lactic acidosis and dehydration. Age was not associated with saliva pH, which is consistent with the findings of previous authors who have reported that saliva pH increases between 8 and 50 days of age, but not between 8 and 36 days of age (36).



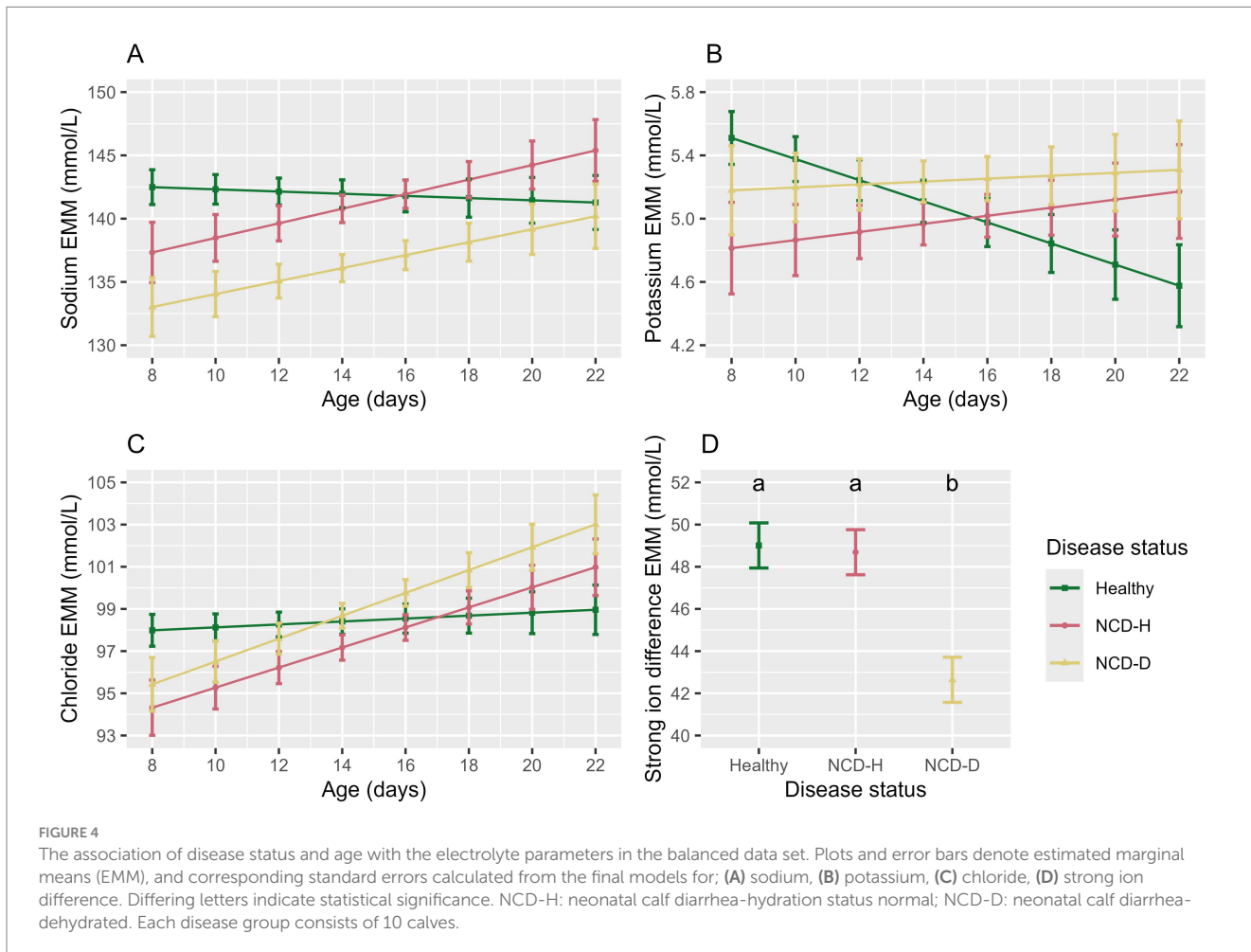
Another factor to consider is that the saliva pH may be altered by the nutritional status of the calf which may become inappetent with NCD, further research could analyze the relationship between milk intake and saliva pH.

Hematocrit is traditionally used to measure dehydration. The hematocrit results seen in this study were as expected, with hemoconcentration evident in the dehydrated calves. Hematocrit is an important prognostic indicator, and has been shown to be higher on admission to hospital in diarrheic calves that died, when compared to those that survived (54). Dehydration in this study was classified using the return of the skin tent as it has been shown to be associated with changes in hydration status in the absence of a change in hematocrit or total protein in young calves (38). Belgian Blue cows have been shown to have a higher hematocrit than Holstein Friesian cows (55), which is consistent with the increased hematocrit in beef-sired calves in this study. However, this difference was not seen in Dillane et al. (56) who compared the hematocrit of dairy and beef calves. The lower hematocrit in males calves in this study is consistent with that of previous studies for both beef (56) and Holstein calves (57).

The PTP can be used to aid in interpretation of hematocrit results. PTP in this study was lower in hydrated calves with diarrhea but not in dehydrated calves with diarrhea. This reduction in the PTP of diarrheic calves is consistent with the results of Kabu et al. (58) and

Hildebrandt et al. (59), however neither study differentiated between dehydrated and non-dehydrated calves. The serum protein results however are slightly contradictory with no affect of disease on STP, globulin or IgG seen. Albumin levels were higher in the dehydrated calves. It is not clear why these findings are contradictory and further analysis of serum proteins in a greater number of calves is required to further understand these results.

The reduction in potassium in calves with diarrhea but not dehydration was an interesting finding, although it was not seen in the pairwise comparisons. While hypokalemia has been identified in calves undergoing treatment for dehydration (60), all samples were taken in the morning prior to any treatment taking place. Much of the literature in this area relates to hospitalized calves and thus pre-selects severe cases, e.g., Trefz et al. (61) or experimentally induced metabolic acidosis to test the efficacy of treatments, e.g., Schwedhelm et al. (62). In contrast, this study focused on spontaneous occurring disease of all severities by following calves in their normal environment. Previous research has found total protein to be weakly correlated with potassium ($r_p = 0.46$) so it may be that this finding is linked to the reduction in protein seen in the calves with diarrhea but not dehydration (61). Further work on calves with mild-to-moderate disease is needed to explore the significance of these potassium results. The association between increasing age and reducing serum potassium concentration is consistent with the findings of Dillane et al. (56).



Strong ion difference (SID) was lower in calves with diarrhea and dehydration. This means that calves have lost a greater number of cations when compared to anions and is indicative of metabolic acidosis. Many studies have used measures such as anion gap and extracellular base excess to examine acid–base disturbances in calves (32). This was not possible in this study due to budgetary constraints. SID was chosen for use in this study, as it is commonly used as a proxy for acid–base balance and has been associated with changes in both the suckle reflex and posture in young calves with NCD (32). SID has also been shown to be correlated with lactate in calves with diarrhea and no other clinical signs (63).

Some studies have not clearly differentiated between diarrheic calves with or without dehydration. Hildebrandt et al. (59) found no changes in sodium, potassium or chloride in diarrheic calves, but found a difference in the anion base excess. Interestingly, Trefz et al. (17) found an association between survival and sodium and chloride concentrations, however clinical signs had a greater association with survival. Another study found that calves with diarrhea that died had a higher hematocrit, higher levels of sodium and chloride but no difference in SID or potassium (54). Sayers et al. (34) used a clinical assessment scoring system designed by the research farm and its veterinary surgeons that included signs of dehydration. In that study, disease severity was significantly correlated with sodium, SID, total hemoglobin, and blood pH, but not with potassium or chloride. This concurred with the results for

hematocrit, SID, potassium and chloride of this study, but not those of sodium.

In addition to differences in disease severity between studies, NCD is caused by a range of pathogens often acting in concert, which may impact differently on the anion gap and extracellular base excess of calves between studies. In this study the predominate pathogen was *Cryptosporidium* (62 cases), with *Bovine Rotavirus*, *Bovine Coronavirus* and *E. coli* K99, also being identified. Fourteen cases had multiple pathogens identified. *E. coli* K99 was not thought to be significant as the calf in question was 18 days old and this pathogen usually affects calves less than 4 days of age (64). *Cryptosporidium* infection causes villous atrophy in the small intestine leading to malabsorption, with increased secretion of chloride and bicarbonate and reduced absorption of sodium chloride (65). *Bovine Rotavirus* and *Bovine Coronavirus* also cause damage to the villi with decreased absorption of sodium chloride and water (66). The number of calves in this study does not allow for analysis of the biochemical differences between pathogens, or comparisons of pathogen prevalence between the dehydrated and normally hydrated NCD groups.

The NCD was associated with saliva pH in this study. The Lin's concordance correlation between either hematocrit or SID and saliva pH was negligible, however. This suggests that the mechanism by which saliva pH changes in NCD requires further research. It is possible that saliva pH can be used “pen side” by veterinary surgeons or farm staff when assessing a calf on farm to allow them to choose an

TABLE 4 The association of neonatal calf diarrhea and hydration status with serum electrolytes artificially reared calves.

Variable	Factor	Level	Number of calves ^b	Estimate	Confidence interval	Degrees of freedom	t-value	P-value
Sodium (mmol/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H ^a	10	-10.46	-21.65 - 0.73		-2.648	0.066
		NCD-D ^a	10	-14.26	-25.38 - -3.14		-1.929	0.014
	Age		30	-0.09	-0.49 - 0.32	1	-0.444	0.661
	Age*Disease							0.120
Potassium (mmol/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H	10	-1.44	-2.80 - -0.07		-2.177	0.040
		NCD-D	10	-0.94	-2.29 - 0.41		-1.455	0.164
	Age		30	-0.07	-0.12 - -0.02	1	-2.187	0.010
	Age*Disease							0.079
Chloride (mmol/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H	10	-6.92	-13.07 - -0.77		-2.321	0.003
		NCD-D	10	-6.34	-12.45 - -0.23		-2.143	0.042
	Age		30	0.07	-0.15 - 0.29	1	0.650	0.522
	Age*Disease							0.039
Strong ion difference (mmol/L)	Disease	Healthy	10	Reference	Reference	2	Reference	Reference
		NCD-H	10	-0.32	-3.42 - 2.78		-0.212	0.834
		NCD-D	10	-6.30	-9.47 - 3.27		-4.001	<0.001

The results shown are from the final linear models. Variables shown in bold have $p < 0.05$.

^aNCD-H: neonatal calf diarrhea-hydration status normal; NCD-D: neonatal calf diarrhea-dehydrated.

^bAs each calf was only included once in this dataset the number of calves and the number of calf days are equivalent.

appropriate course of treatment. This would allow prompt and appropriate treatment to be administered and thus reduce the risk of mortality. Historically, assessment of hydration status or acid-base balance has required access to equipment that is not possible to carry on to commercial farms. The pH meter used in this study was designed to be portable. There is a need for further research as to whether saliva pH cut-offs can be ascertained at which, e.g., intravenous fluids are indicated rather than oral rehydration solution. Saliva pH could be added to decision trees such as that developed by Trefz et al. (67).

The methods described in this study require a centrifuge for saliva separation and had a one-minute sampling time. This study was designed as proof of concept, and this method would not be practical for on-farm use. In future, it may be possible to measure saliva pH at the milk feeding teat to allow early detection of disease. A further key area of development will be to explore the within-individual variation of saliva pH as this will have a large effect on its use for daily monitoring. Early detection allows early intervention with oral rehydration solution, this will not only improve calf welfare but will improve outcomes and may reduce the need for antibiotics. The automatic measuring of saliva pH may also allow it to be combined with other measures such as feeding behavior to increase accuracy. Accuracy is essential in these systems to prevent the labor cost of unnecessary interventions and incorrect alerts.

5 Conclusion

Reductions in saliva pH are associated with NCD, but further work is required to ascertain the physiological mechanism. Work

including evaluating a greater number of dehydrated calves is needed to ascertain whether saliva pH can differentiate between different levels of disease severity. Saliva conductivity was not associated with NCD, regardless of hydration status. Changes in blood parameters of dehydrated calves were consistent with previous studies. Further investigation of changes in potassium and serum proteins in NCD with and without dehydration is warranted. Saliva pH has potential as a novel indicator of diarrhea in calves.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Animal Experiment Committee, Scotland's Rural College. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

BR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. AC: Conceptualization, Methodology, Supervision, Writing – review & editing. MH: Conceptualization,

Methodology, Project administration, Supervision, Writing – review & editing. C-AD: Conceptualization, Methodology, Supervision, Writing – review & editing. AM: Funding acquisition, Resources, Writing – review & editing. EB: Investigation, Writing – review & editing. CM: Conceptualization, Methodology, Supervision, Writing – review & editing.

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References

1. Palczynski LJ, Bleach ECL, Brennan ML, Robinson PA. Stakeholder perceptions of disease Management for Dairy Calves: "It's just little things that make such a big difference". *Animals*. (2021) 11:2829. doi: 10.3390/ani11102829
2. Johnson KF, Chancellor N, Burn CC, Wathes DC. Prospective cohort study to assess rates of contagious disease in pre-weaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. *Vet Rec Open*. (2017) 4:e000226. doi: 10.1136/vetreco-2017-000226
3. Gomez DE, Arroyo LG, Renaud DL, Viel L, Weese JS. A multidisciplinary approach to reduce and refine antimicrobial drugs use for Diarrhoea in dairy calves. *Vet J*. (2021) 274:105713. doi: 10.1016/j.tvjl.2021.105713
4. Urie NJ, Lombard JE, Shivley CB, Koprak CA, Adams AE, Earleywine TJ, et al. Preweaned heifer management on us dairy operations: part V. Factors associated with morbidity and mortality in Preweaned dairy heifer calves. *J Dairy Sci*. (2018) 101:9229–44. doi: 10.3168/jds.2017-14019
5. Johnson KF, Nair RV, Wathes DC. Comparison of the effects of high and low Milk-replacer feeding regimens on health and growth of crossbred dairy heifers. *Anim Prod Sci*. (2019) 59:1648–59. doi: 10.1071/AN18432
6. Olson A, Sischo WM, Berge ACB, Adams-Progar A, Moore DA. A retrospective cohort study comparing dairy calf treatment decisions by farm personnel with veterinary observations of clinical signs. *J Dairy Sci*. (2019) 102:6391–403. doi: 10.3168/jds.2018-15623
7. McCarthy HR, Cantor MC, Lopez AJ, Pineda A, Nagorske M, Renaud DL, et al. Effects of supplementing colostrum beyond the first day of life on growth and health parameters of Preweaning Holstein heifers. *J Dairy Sci*. (2024) 107:3280–91. doi: 10.3168/jds.2023-23649
8. Renaud DL, Rot C, Marshall J, Steele MA. The effect of *Cryptosporidium parvum*, rotavirus, and coronavirus infection on the health and performance of male dairy calves. *J Dairy Sci*. (2021) 104:2151–63. doi: 10.3168/jds.2020-19215
9. Johnson KF, Chancellor N, Burn CC, Wathes DC. Analysis of pre-weaning feeding policies and other risk factors influencing growth rates in calves on 11 commercial dairy farms. *Animal*. (2018) 12:1413–23. doi: 10.1017/S1751731117003160
10. Abuelo A, Cullens F, Brester JL. Effect of Preweaning disease on the reproductive performance and first-lactation Milk production of heifers in a large dairy herd. *J Dairy Sci*. (2021) 104:7008–17. doi: 10.3168/jds.2020-19791

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Conflict of interest

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Supplementary material

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11. Goh N, House J, Rowe S. Retrospective cohort study investigating the relationship between diarrhea during the Preweaning period and subsequent survival, health, and production in dairy cows. *J Dairy Sci*. (2024) 107:9752–61. doi: 10.3168/jds.2023-24544
12. Bach A. Associations between several aspects of heifer development and dairy cow survivability to second lactation. *J Dairy Sci*. (2011) 94:1052–7. doi: 10.3168/jds.2010-3633
13. Shaw HJ, Innes EA, Morrison LJ, Katzer F, Wells B. Long-term production effects of clinical cryptosporidiosis in neonatal calves. *Int J Parasitol*. (2020) 50:371–6. doi: 10.1016/j.ijpara.2020.03.002
14. Scott K, Kelton DE, Duffield TF, Renaud DL. Risk factors identified on arrival associated with morbidity and mortality at a grain-fed veal facility: a prospective, Single-Cohort Study. *J Dairy Sci*. (2019) 102:9224–35. doi: 10.3168/jds.2019-16829
15. Renaud DL, Overton MW, Kelton DE, LeBlanc SJ, Dhuyvetter KC, Duffield TF. Effect of health status evaluated at arrival on growth in Milk-fed veal calves: a prospective single cohort study. *J Dairy Sci*. (2018) 101:10383–90. doi: 10.3168/jds.2018-14960
16. Schinwald M, Creutzinger K, Keunen A, Winder CB, Haley D, Renaud DL. Predictors of diarrhea, mortality, and weight gain in male dairy calves. *J Dairy Sci*. (2022) 105:5296–309. doi: 10.3168/jds.2021-21667
17. Trefz FM, Lorenz I, Lorch A, Constable PD. Clinical signs, profound Acidemia, hypoglycemia, and hypernatremia are predictive of mortality in 1,400 critically ill neonatal calves with diarrhea. *PLoS One*. (2017) 12:e0182938. doi: 10.1371/journal.pone.0182938
18. Goharshahi M, Azizzadeh M, Lidauer L, Steininger A, Kicking F, Ohlschuster M, et al. Monitoring selected behaviors of calves by use of an ear-attached accelerometer for detecting early indicators of diarrhea. *J Dairy Sci*. (2021) 104:6013–9. doi: 10.3168/jds.2020-18989
19. Sutherland MA, Lowe GL, Huddart FJ, Waas JR, Stewart M. Measurement of dairy calf behavior prior to onset of clinical disease and in response to disbudding using automated calf feeders and accelerometers. *J Dairy Sci*. (2018) 101:8208–16. doi: 10.3168/jds.2017-14207
20. Scoley G, Gordon A, Morrison S. Using non-invasive monitoring technologies to capture behavioural, physiological and health responses of dairy calves to different nutritional regimes during the first ten weeks of life. *Animals*. (2019) 9:760. doi: 10.3390/ani9100760

21. Svensson C, Jensen MB. Short communication: identification of diseased calves by use of data from automatic Milk feeders. *J Dairy Sci.* (2007) 90:994–7. doi: 10.3168/jds.S0022-0302(07)71584-9
22. Conboy MH, Winder CB, Cantor MC, Costa JHC, Steele MA, Medrano-Galarza C, et al. Associations between feeding behaviors collected from an automated Milk feeder and neonatal calf diarrhea in group housed dairy calves: a case-control study. *Animals.* (2022) 12:170. doi: 10.3390/ani12020170
23. Lowe GL, Sutherland MA, Waas JR, Schaefer AL, Cox NR, Stewart M. Physiological and behavioral responses as indicators for early disease detection in dairy calves. *J Dairy Sci.* (2019) 102:5389–402. doi: 10.3168/jds.2018-15701
24. Munoz CX, Johnson EC, Demartini JK, Huggins RA, McKenzie AL, Casa DJ, et al. Assessment of hydration biomarkers including salivary osmolality during passive and active dehydration. *Eur J Clin Nutr.* (2013) 67:1257–63. doi: 10.1038/ejcn.2013.195
25. Taylor NA, van den Heuvel AM, Kerry P, McGhee S, Peoples GE, Brown MA, et al. Observations on saliva osmolality during progressive dehydration and partial rehydration. *Eur J Appl Physiol.* (2012) 112:3227–37. doi: 10.1007/s00421-011-2299-z
26. Ring M, Lohmueller C, Rauh M, Mester J, Eskofier BM. Salivary markers for quantitative dehydration estimation during physical exercise. *IEEE J Biomedical Health Informat.* (2017) 21:1306–14. doi: 10.1109/JBHI.2016.2598854
27. Owen JA, Fortes MB, Ur Rahman S, Jibani M, Walsh NP, Oliver SJ. Hydration marker diagnostic accuracy to identify mild intracellular and extracellular dehydration. *Int J Sport Nutr Exer Metabol.* (2019) 29:604–11. doi: 10.1123/ijsem.2019-0022
28. Lu YP, Huang JW, Lee IN, Weng RC, Lin MY, Yang JT, et al. A portable system to monitor saliva conductivity for dehydration diagnosis and kidney healthcare. *Sci Rep.* (2019) 9:14771. doi: 10.1038/s41598-019-51463-8
29. Lei MC, Félix L, Cardoso R, Monteiro SM, Silva S, Venâncio C. Non-invasive biomarkers in saliva and eye infrared thermography to assess the stress response of calves during transport. *Animals.* (2023) 13:2311. doi: 10.3390/ani13142311
30. Berteselli GV, Filipe J, Martelli A, Vezzaro G, Canali E, Dall'Arca P. Salivary IgG and Iga in newborn calves and the possible role in the assessment of passive immunity transfer. *Front Vet Sci.* (2024) 11:11. doi: 10.3389/fvets.2024.1383379
31. Lorenz I. D-lactic acidosis in calves. *Vet J.* (2009) 179:197–203. doi: 10.1016/j.tvjl.2007.08.028
32. Gomez DE, Lofstedt J, Stampfli HR, Wichtel M, Muirhead T, McClure JT. Contribution of unmeasured anions to acid-base disorders and its association with altered demeanor in 264 calves with neonatal diarrhea. *J Vet Intern Med.* (2013) 27:1604–12. doi: 10.1111/jvim.12193
33. Groutides CP, Michell AR. Changes in plasma composition in calves surviving or dying from Diarrhoea. *Br Vet J.* (1990) 146:205–10. doi: 10.1016/s0007-1935(11)80003-5
34. Sayers RG, Kennedy A, Krump L, Sayers GP, Kennedy E. An observational study using blood gas analysis to assess neonatal calf diarrhea and subsequent recovery with a European Commission-compliant Oral electrolyte solution. *J Dairy Sci.* (2016) 99:4647–55. doi: 10.3168/jds.2015-10600
35. Sánchez J, Fuentes N, Ibañez-López FJ, López-García I, Gutiérrez AM. A multi-herd study shows that saliva is more than a reflection of serum biomarkers in pigs. *Animal.* (2021) 15:100413. doi: 10.1016/j.animal.2021.100413
36. Schwarzkopf S, Kinoshita A, Hüther L, Salm L, Kehraus S, Südekum K-H, et al. Weaning age influences indicators of rumen function and development in female Holstein calves. *BMC Vet Res.* (2022) 18:102. doi: 10.1186/s12917-022-03163-1
37. McGuirk SM. Disease Management of Dairy Calves and Heifers. *Vet Clin N Am Food Anim Pract.* (2008) 24:139–53. doi: 10.1016/j.cvfa.2007.10.003
38. Kells NJ, Beausoleil NJ, Johnson CB, Chambers JP, O'Connor C, Webster J, et al. Indicators of dehydration in healthy 4- to 5-day-old dairy calves deprived of feed and water for 24 hours. *J Dairy Sci.* (2020) 103:11820–32. doi: 10.3168/jds.2020-18743
39. Sirois M, Hendrix CM. Laboratory procedures for veterinary technicians. 6th ed. St. Louis, Missouri: Elsevier/Mosby (2014). 440 p p.
40. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the Tidyverse. *J Open Source Softw.* (2019) 4:1686. doi: 10.21105/joss.01686
41. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing (2021).
42. Hartig F. Dharma: residual diagnostics for hierarchical (multi-level/mixed) regression models. (2022). Available at: <https://CRAN.R-project.org/package=DHARMA> (Accessed February 27, 2024).
43. Kuznetsova A, Brockhoff PB, Christensen RHB. LmerTest package: tests in linear mixed effects models. *J Stat Softw.* (2017) 82:1–26. doi: 10.18637/jss.v082.i13
44. Lenth RV. Emmeans: estimated marginal means, aka least-squares means. (2024). Available at: <https://CRAN.R-project.org/package=emmeans> (Accessed February 27, 2024).
45. Wickham H. Ggplot2: elegant graphics for data analysis. 2nd ed. Springer-Verlag New York: Cham (2016).
46. Signorelli A. DescTools: tools for descriptive statistics. Available at: <https://andrisignorelli.github.io/DescTools/> (Accessed October 24, 2024).
47. Constable PD. Clinical assessment of Acid-Base status: strong ion difference theory. *Vet Clin N Am Food Anim Pract.* (1999) 15:447–71. doi: 10.1016/S0749-0720(15)30158-4
48. Yoo DW, Lee SM, Moon SY, Kim IS, Chang CL. Evaluation of conductivity-based osmolality measurement in urine using the Sysmex Uf5000. *J Clin Lab Anal.* (2021) 35:e23586. doi: 10.1002/jcla.23586
49. Ely BR, Cheuvront SN, Kenefick RW, Sawka MN. Limitations of salivary osmolality as a marker of hydration status. *Med Sci Sports Exerc.* (2011) 43:1080–4. doi: 10.1249/MSS.0b013e3182058643
50. Humphrey SP, Williamson RT. A review of saliva: Normal composition, flow, and function. *J Prosthet Dent.* (2001) 85:162–9. doi: 10.1067/mpr.2001.113778
51. Herdt TH. 29 – secretions of the gastrointestinal tract In: BG Klein, editor. Cunningham's textbook of veterinary physiology. 6th ed. St. Louis (MO): W.B. Saunders (2020). 307–15.
52. Meganck V, Hoflack G, Opsomer G. Advances in prevention and therapy of neonatal dairy calf Diarrhoea: a systematic review with emphasis on colostrum management and fluid therapy. *Acta Vet Scand.* (2014) 56:75. doi: 10.1186/s13028-014-0075-x
53. Lorenz I. Influence of D-lactate on metabolic acidosis and on prognosis in neonatal calves with Diarrhoea. *J Veterinary Med Ser A.* (2004) 51:425–8. doi: 10.1111/j.1439-0442.2004.00662.x
54. Ekinci G, Tüfekçi E, Cissé Y, Bekdik IK, Onmaz AC, Aslan Ö, et al. Chloride and lactate as prognostic indicators of calf diarrhea from eighty-nine cases. *J Vet Sci.* (2024) 25:e38. doi: 10.4142/jvs.23155
55. Guyot H, Legroux D, Eppe J, Bureau F, Cannon L, Ramery E. Hematologic and serum biochemical characteristics of Belgian blue cattle. *Vet Sci.* (2024) 11:222. doi: 10.3390/vetsci11050222
56. Dillane P, Krump L, Kennedy A, Sayers RG, Sayers GP. Establishing blood gas ranges in healthy bovine neonates differentiated by age, sex, and breed type. *J Dairy Sci.* (2018) 101:3205–12. doi: 10.3168/jds.2017-13445
57. Panousis N, Siachos N, Kitkas G, Kalaitzakis E, Kritsepi-Konstantinou M, Valergakis GE. Hematology reference intervals for neonatal Holstein calves. *Res Vet Sci.* (2018) 118:1–10. doi: 10.1016/j.rvsc.2018.01.002
58. Kabu M, Elitok B, Kucukurt I. Detection of serum amyloid-a concentration in the calf clinically diagnosed with pneumonia, enteritis and pneumoenteritis. *Cienc Rural.* (2016) 46:293–9. doi: 10.1590/0103-8478cr20150571
59. Hildebrandt T, Scheuch E, Weitschies W, Schneider F, Grimm M, Bachmann L, et al. Abomasal emptying rate of diarrhoeic and healthy suckling calves fed with Oral rehydration solutions. *J Anim Physiol Anim Nutr.* (2020) 104:462–9. doi: 10.1111/jpn.13306
60. Trefz FM, Lorch A, Zitzl J, Kutschke A, Knubben-Schweizer G, Lorenz I. Risk factors for the development of hypokalemia in neonatal diarrheic calves. *J Vet Intern Med.* (2015) 29:688–95. doi: 10.1111/jvim.12541
61. Trefz FM, Constable PD, Sauter-Louis C, Lorch A, Knubben-Schweizer G, Lorenz I. Hyperkalemia in neonatal diarrheic calves depends on the degree of dehydration and the cause of the metabolic acidosis but does not require the presence of Acidemia. *J Dairy Sci.* (2013) 96:7234–44. doi: 10.3168/jds.2013-6945
62. Schwedhelm L, Kirchner D, Klaus B, Bachmann L. Experimentally induced hyperchloremic and dl-lactic acidosis in calves: an attempt to study the effects of Oral rehydration on acid-Base status. *J Dairy Sci.* (2013) 96:2464–75. doi: 10.3168/jds.2012-6077
63. Bednarski M, Kupczyński R. Analysis of Acid-Base disorders in calves with lactic acidosis using a classic model and strong ion approach. *Turk J Vet Anim Sci.* (2015) 39:615–20. doi: 10.3906/vet-1502-42
64. Foster DM, Smith GW. Pathophysiology of diarrhea in calves. *Vet Clin N Am Food Anim Pract.* (2009) 25:13–36. doi: 10.1016/j.cvfa.2008.10.013
65. Wyatt CR, Riggs MW, Fayer R. Cryptosporidiosis in neonatal calves. *Vet Clin N Am Food Anim Pract.* (2010) 26:89–103. doi: 10.1016/j.cvfa.2009.10.001
66. Geletu US, Usmael MA, Bari FD. Rotavirus in calves and its zoonotic importance. *Vet. Med Int.* (2021) 2021:6639701–18. doi: 10.1155/2021/6639701
67. Trefz FM, Lorch A, Feist M, Sauter-Louis C, Lorenz I. Construction and validation of a decision tree for treating metabolic acidosis in calves with neonatal diarrhea. *BMC Vet Res.* (2012) 8:238. doi: 10.1186/1746-6148-8-238
68. McGuirk SM. Calf health scoring chart. In: calf_health_scoring_chart, editor. School of Veterinary Medicine, Wisconsin-Madison University (2015). Available at: https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf (Accessed May 01, 2024)

4.7 Additional discussion & limitations

As discussed in Section 1.5.3 there are several methods for diagnosis of NCD. In this study faecal scoring using the scale by McGuirk (2015) and a cleanliness score of the calf's hind legs, perineum, and tail (CLEAN) were used. The faeces score has recently been shown to have very good intra- and inter- rater reliability (Ramos *et al.*, 2025). However, a previous study found interrater reliability to only be moderate (Renaud *et al.*, 2020). Two studies have found that faecal score is associated with faecal dry matter with the four levels being significantly different from each other (Renaud *et al.*, 2020, Bellosa *et al.*, 2011)

The CLEAN scale used in this study was based on a previous scale originally used by Volac and promoted by AHDB (Agriculture and Horticulture Development Board *et al.*, 2018) and has not been scientifically validated. However, moderate interrater agreement was found for the similar rear component of the calf hygiene score by Kellerman *et al.* (2020) (Kappa = 0.70, 0.64 - 0.77). This is further supported by Ramos *et al.* (2025), where both intra-and inter-rater agreement of the rear component of the Kellerman *et al.* (2020) scoring system was very good. The other components of the calf hygiene score by Kellerman *et al.* (2020) were not considered in this study as it was felt that cleanliness of the belly and side of the calf was more likely to reflect the health status of the group than the individual. The CLEAN scale used was modified to a scale of 0 to 3, rather than to 0 to 2. This is as experience during the pilot trial showed that calves were scored a one on the 0 to 2 scale due to dried faeces, thus an additional level separating out dry contamination from wet contamination was introduced (see Table 4.1 above).

A limitation of this study is that the skin tent test for dehydration, only has moderate interrater reliability (Ramos *et al.*, 2025). However, in contrast Kells *et al.* (2020) and Constable *et al.* (1998) found that skin tent elasticity, enophthalmos, and capillary refill time were all predictors of dehydration. This limitation was partly controlled for by me carrying out 85% of the scoring, however intra-rater reliability is only moderate therefore reliability is still a concern. Due to daily scoring being an important part of the study design, it was not possible for me to carry out all the

scoring. The remaining 15% of calf days were scored by three technicians, all of whom regularly accompanied me during scoring and sampling, this should have facilitated a consistent approach. If I were to carry this study out again then I would formalise this approach by training with all technicians prior to commencing, followed by the technicians and myself independently scoring a new pen and then calculating interrater reliability. However, the low prevalence of dehydration means that it may not be present on the day training is carried out, thus preventing the calculation of interrater reliability for skin tent assessment. Interestingly, Ramos *et al.* (2025) found that the inter-and intra-observer reliability of enophthalmos was very good. I had assumed that skin tent return time would be easier to measure, I may however have been better off choosing enophthalmos. In conclusion, while this study showed some promise for saliva pH the mechanism is not clear at present and there are several methodological concerns that should be addressed in further work.

4.8 Bibliography

AGRICULTURE AND HORTICULTURE DEVELOPMENT BOARD, POTTER, T. & PICK, S. 2018. Better Returns from calf rearing.

<https://media.ahdb.org.uk/media/Default/Imported%20Publication%20Docs/Better%20returns%20from%20calf%20rearing.pdf>: AHDB.

BEDNARSKI, M. & KUPCZYŃSKI, R. 2015. Analysis of acid–base disorders in calves with lactic acidosis using a classic model and strong ion approach. *Turkish Journal of Veterinary and Animal Sciences*, 39, 615-620.

BELLOSA, M. L., NYDAM, D. V., LIOTTA, J. L., JENNIFE, A. Z., LINDEN, T. C. & BOWMAN, D. D. 2011. A Comparison of Fecal Percent Dry Matter and Number of *Cryptosporidium parvum* Oocysts Shed to Observational Fecal Consistency Scoring in Dairy Calves. *Journal of Parasitology*, 97, 349-351.

CONSTABLE, P. D., WALKER, P. G., MORIN, D. E. & FOREMAN, J. H. 1998. Clinical and laboratory assessment of hydration status of neonatal calves with diarrhea. *Journal of the American Veterinary Medical Association*, 212, 991-996.

HAMADA, A. M., SABRY, M. H., SAMY, A. & ALANSARY, A. 2013. Salivary Anion Gap and Na/K ratio as Predictive of Acid-Base Balance after Open Heart Surgery. *Life Science Journal-Acta Zhengzhou University Overseas Edition*, 10, 1045-1049.

- JOHNSON, K. F., NAIR, R. V. & WATHES, D. C. 2019. Comparison of the effects of high and low milk-replacer feeding regimens on health and growth of crossbred dairy heifers. *Animal Production Science*, 59, 1648-1659.
- KELLERMANN, L. M., RIEGER, A., KNUBBEN-SCHWEIZER, G. & METZNER, M. 2020. Short communication: Design and validation of a hygiene score for calves. *Journal of Dairy Science*, 103, 3622-3627.
- KELLS, N. J., BEAUSOLEIL, N. J., JOHNSON, C. B., CHAMBERS, J. P., O'CONNOR, C., WEBSTER, J., LAVEN, R. & COGGER, N. 2020. Indicators of dehydration in healthy 4- to 5-day-old dairy calves deprived of feed and water for 24 hours. *Journal of Dairy Science*, 103, 11820-11832.
- LU, Y. P., HUANG, J. W., LEE, I. N., WENG, R. C., LIN, M. Y., YANG, J. T. & LIN, C. T. 2019. A Portable System to Monitor Saliva Conductivity for Dehydration Diagnosis and Kidney Healthcare. *Scientific Reports*, 9, 14771.
- MCGUIRK, S. M. 2015. Calf Health Scoring Chart *In: CALF_HEALTH_SCORING_CHART* (ed.). https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf: School of Veterinary Medicine, Wisconsin-Madison University.
- MUNOZ, C. X., JOHNSON, E. C., DEMARTINI, J. K., HUGGINS, R. A., MCKENZIE, A. L., CASA, D. J., MARESH, C. M. & ARMSTRONG, L. E. 2013. Assessment of hydration biomarkers including salivary osmolality during passive and active dehydration. *European Journal of Clinical Nutrition*, 67, 1257-63.
- RAMOS, J. S., VILLETAZ ROBICHAUD, M., DUBUC, J., SANTACHI, D., ROY, J.-P., FECTEAU, G. & BUCZINSKI, S. 2025. Utility and reliability of various on-farm welfare and health indicators for preweaning dairy calves. *Journal of Dairy Science*, 108, 11493-11507.
- RENAUD, D. L., BUSS, L., WILMS, J. N. & STEELE, M. A. 2020. Technical note: Is fecal consistency scoring an accurate measure of fecal dry matter in dairy calves? *Journal of Dairy Science*, 103, 10709-10714.
- SUZUKI, D., YAMADA, S. I., SAKURAI, A., KARASAWA, I., KONDO, E., SAKAI, H., TANAKA, H., SHIMANE, T. & KURITA, H. 2020. Correlations between the properties of saliva and metabolic syndrome: A prospective observational study. *Medicine (Baltimore)*, 99, e23688.
- TAYLOR, N. A., VAN DEN HEUVEL, A. M., KERRY, P., MCGHEE, S., PEOPLES, G. E., BROWN, M. A. & PATTERSON, M. J. 2012. Observations on saliva osmolality during progressive dehydration and partial rehydration. *European Journal of Applied Physiology*, 112, 3227-37.
- TREFZ, F. M., CONSTABLE, P. D., SAUTER-LOUIS, C., LORCH, A., KNUBBEN-SCHWEIZER, G. & LORENZ, I. 2013. Hyperkalemia in neonatal diarrheic calves

depends on the degree of dehydration and the cause of the metabolic acidosis but does not require the presence of acidemia. *Journal of Dairy Science*, 96, 7234-7244.

Chapter 5 The association of feeding behaviours and activity parameters with neonatal calf diarrhoea with or without dehydration in pre-weaned, group-housed, dairy-bred calves.

5.0 Preface

In the previous chapter, a novel biomarker for the physiological changes in calves with neonatal calf diarrhoea was identified. In chapter 3, associations between bovine respiratory disease and calf behaviour were explored. This chapter will explore the association between neonatal calf diarrhoea and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.

5.1 Abstract

Neonatal calf diarrhoea (NCD) is a prevalent and significant disease in young dairy-bred calves. Dehydration may present in the more severe cases and increases the risk of mortality. Previous research has shown that the feeding behaviours and activity variables of young calves change when they develop NCD. Technology allows us to monitor calves around the clock and may therefore give us an opportunity to detect disease earlier. This study aimed to characterise the association between NCD with and without dehydration and activity and milk feeding variables.

One-hundred and forty-one group housed calves were monitored from seven days until twenty-five days of age. Calves were health scored daily including cleanliness, hydration status, and where necessary faecal consistency. Automatic milk feeders recorded milk feeding behaviours and activity variables were recorded by triaxial accelerometers. Generalised linear mixed modelling approaches were used to analyse the data other than mean milk meal size which was analysed using a cumulative link mixed model.

Calves with both classes of NCD were less active in each standing bout and less likely to consume their milk allowance each day than healthy calves. Calves with NCD and a normal hydration status had fewer, longer lying bouts and more standing bouts than healthy calves. In autumn, calves with NCD regardless of hydration status were less active and drank more slowly than their healthy counterparts. Calves with NCD and dehydration had longer visits to milk than either of the other groups in autumn. Diarrheic calves that were normally hydrated visited the milk less often in autumn and, visited the milk for longer in all three seasons when compared to their healthy counterparts. Other factors including season, age and sire-breed type were also associated with lying, standing, activity and milk feeding variables.

While changes in activity were detected in calves with NCD, only the number of milk visits in autumn was different between dehydrated and non-dehydrated calves, probably due to the low number of dehydrated calves in the dataset. There

was a high rate of data loss in this study and this needs to be addressed to allow effective disease detection tools to be developed.

5.2 Introduction

Diarrhoea or “scours” in pre-weaned calves is clinically termed neonatal calf diarrhoea (NCD) and is considered a major concern by both farmers and veterinary surgeons (Palczynski *et al.*, 2021). In the previous chapter potential physiological indicators of NCD were explored. In this chapter behavioural indicators of NCD will be explored. Neonatal calf diarrhoea is one of the two most prevalent diseases in pre-weaned, dairy-bred calves. A variety of pathogens are responsible for NCD and infection with multiple pathogens is frequent (Jessop *et al.*, 2024). Estimates of on farm prevalence include 19.6% in Germany (Dachrodt *et al.*, 2021) and 17% in Sweden (Medrano-Galarza *et al.*, 2018b). Incidence rate in the UK has been estimated at 48 per 100 calves in the first 9 weeks of life (range: 24 – 74 per 100 calves) (Johnson *et al.*, 2017). Neonatal calf diarrhoea can lead to several complications, including dehydration and acidosis and can be fatal, with studies estimating that 8.5% of calves with NCD will die (Urie *et al.*, 2018). Dehydration develops due to a reduction in the absorption of water, an increase in intestinal secretion and an increased passage rate through the intestine (Berchtold, 2009). Severe dehydration will increase the risk of mortality (Trefz *et al.*, 2017).

Neonatal calf diarrhoea can have long-term consequences for the affected calves. Neonatal calf diarrhoea is associated with a reduction in growth rates (Renaud *et al.*, 2021, Abuelo *et al.*, 2021) and may have a long-term effect on heifers including an increase in the age at first calving and a reduction in first lactation milk yield (Abuelo *et al.*, 2021). However, this is not supported in all the literature (Boccardo *et al.*, 2019).

Early detection of disease is vital to reduce the welfare impact of disease on the calf. Calves treated with a non-steroidal anti-inflammatory (NSAID) when they developed diarrhoea were more likely to consume their milk allowance and gained weight faster than those treated by a placebo (Todd *et al.*, 2010). Thus, detecting disease early may also improve calf performance.

In the previous chapter, physiological indicators of NCD were explored. In this chapter, possible behavioural indicators of NCD will be explored using both automatic milk feeders and wearable technology. An increased use of group housing and automatic milk feeding on commercial farms provides opportunities to record milk feeding behaviours automatically. Farmers have identified time as a challenge on farm (Russel *et al.*, 2022). Automatic milk feeders reduce labour costs without affecting calf performance (Sinnott *et al.*, 2021). Automatic milk feeders have varying uptake worldwide, with estimates including 16% of Canadian dairy farms (Medrano-Galarza *et al.*, 2017) and 6.6% of Australian dairy farms (Abuelo *et al.*, 2019). One study showed that 2.3% of dairy farms in the UK used automatic milk feeders (Mahendran *et al.*, 2022), however a Northern Ireland study found that 21.2% of farms used an automatic milk feeder (Brown *et al.*, 2021). Automatic methods of disease detection allow for 24-hour monitoring which is not feasible for farm staff. This may be of particular benefit as cattle are a prey species and thus may mask symptoms when staff are in the vicinity.

Previous research has shown a reduction in milk consumed (Sutherland *et al.*, 2018) and a slower drinking speed (Knauer *et al.*, 2017) in calves with diarrhoea, which suggests that there may be behavioural indicators of NCD that may be used to detect the disease. Wearable technologies can also be utilized to record the activity variables of calves, where previous research has used ear (Goharshahi *et al.*, 2021) and leg (Swartz *et al.*, 2020) mounted accelerometers. Changes in behaviours such as the number of lying bouts, (Lowe *et al.*, 2019), lying time (Swartz *et al.*, 2020) and activity index (Guevara-Mann *et al.*, 2023) have been found in calves with diarrhoea.

In this observational study, possible behavioural indicators of NCD in pre-weaned group housed dairy bred calves will be explored using both automatic milk feeders and wearable technology. Whether disease severity is associated with changes in behaviour will be explored by dealing with normally hydrated and dehydrated NCD calves separately. The objective of this study is to understand the association between neonatal calf diarrhoea and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.

5.3 Methods

5.3.1 Animal management

All calves included in this study were kept at the SRUC Dairy Research and Innovation Centre, Crichton Royal Farm, Dumfries, UK under the approval of the Animal Experiment Committee of SRUC (DAI AE 06-2022). Calves were kept in individual hutches from shortly after birth, until moved into the group pen at approximately one week of age. The hutches were bedded with either straw or woodchip. Calves were offered 3 L of acidified milk replacer twice daily (*Maximum +, Carrs Billington Agriculture (Sales) Ltd., Carlisle*, crude protein 24%, crude oils and fats 20%, crude ash 7.5%, calcium 0.9%, sodium 0.5%, phosphorus 0.7%, mixed to 15%). Calves also had free access to water and calf starter nuts (*Ambition calf and Omnigen nuts, Mole Valley Feed Solutions, South Molton*, dry matter 86.2%, crude protein 18%, crude oils and fats 4.6%, crude fibre 9.2%, crude ash 9.2%, Sodium 0.5%, Selenium 0.4 mg/kg, copper 19 mg/kg, Vitamin E 60 mg/kg). Due to a history of severe acute cryptosporidiosis in calves less than 7 days of age, on the advice of the farm's veterinary surgeon, calves received paromomycin sulphate once daily during their time in the hutch (*Parofor, Huvepharma NV, Antwerp, Belgium*, 10 g/calf/day).

Calves were recruited onto the experiment when they moved into the group pens at approximately seven days of age (6-11 days of age, mean 7.1 days of age, median 7 days of age). Calves were moved into a group pen once there was one available, they had reached a minimum of six days of age and had at least one other calf that was ready to move with them. It took six days on average to fill a pen (3 - 10 days). One hundred and forty-one calves were recruited in total. Calves were classed according to their sire's breed type as shown in Table 5.1. Calves were removed from the study after an episode of diarrhoea or at 26 days of age, whichever came first.

Table 5.1 The season recruited, sex and breed of the calves recruited onto the study.

Sire breed type	Sire breed	Sex	Season recruited			Total		
			Autumn	Winter	Summer			
Beef	Aberdeen Angus	Female	18	8	10	36	74	114
		Male	14	14	10	38		
	British Blue	Female	9	3	8	20	35	
		Male	6	4	5	15		
	Beef Shorthorn	Female	2			2	5	
		Male	3			3		
Dairy	Holstein	Female	10	9		19	27	27
		Male	2	2	4	8		
Total			64	40	37			141

The group pens consisted of an igloo (*Holm & Laue, Moorweg 6, 24784 Westerrönfeld, Germany, 3.9 m x 4.4 m, 2.2 m high*) and a covered pen (5.1 m x 5.1 m) and were bedded with straw. There were ten to twelve calves in each pen (stocking density: 3.6 - 4.3 m²/calf). Calves were fed using an automatic milk feeder (*Custom built for this calf unit, BioControl Norway As, Grimstad Gård, Norway*) and had access to seven litres of milk (as above) daily. The day was split into five feeding periods with 1.4 L of milk becoming available at the start of each period, the maximum meal size was 1.4 L and calves who did not drink 7 L by 23:59 had a maximum of 1.4 L carry over to the next day. Calves also had access to *ad libitum* calf starter (as above) and water.

5.3.2 Health monitoring

Calves were scored each day to assess their health status. The Wisconsin calf health score (WCHS) (McGuirk, 2015) was used to assess signs of respiratory disease. The temperature score was recorded separately (Table 5.2), in addition to being included in the cumulative WCHS. The faeces scoring from the WCHS was used to assess consistency of faeces where applicable (Table 5.2). The cleanliness of each calf's hind legs, perineum, and tail were also assessed (referred to hereafter as CLEAN, Table 5.2). Hydration status was assessed using the skin tent elasticity and capillary refill time. Skin tent elasticity was assessed by using a hand to for a tent of skin behind the shoulder and timing how long it returned to normal. The calves' capillary refill time was assessed by pressing on the oral mucosa and timing how long it took for the colour to return to normal. The additional criteria were required as WCHS does not measure dehydration, and faeces may not be seen in group

housed calves. NCD was defined as a faeces score of ≥ 2 or a CLEAN score of ≥ 2 . Dehydration was defined as a return of the skin tent of > 3 seconds. Capillary refill time was disregarded as it was never prolonged in an animal with a return of the skin tent ≤ 3 seconds. Calves with NCD and a normal hydration status were classified as NCD-H for the day of disease detection and the two subsequent days unless dehydration was detected in which case that day was classified as NCD-D.

Table 5.2 The criteria for the tail, perineum and hindleg cleanliness (CLEAN) and faeces scores

Score	Scoring criteria		
	CLEAN	Faeces (McGuirk, 2015)	Temperature ($^{\circ}\text{C}$)
0	Clean calf or with a small amount of dried faeces on tail/perineum/hind legs	Formed faeces	37.8 – 38.2
1	A large amount of dried faeces or some pasty faeces on tail/perineum/hind legs	Pasty faeces	38.3 – 38.8
2	Wet faeces on tail/perineum/hind legs	Loose faeces that did not sift through bedding	38.9 – 39.4
3	A very wet tail/perineum or a large amount of faeces on tail/perineum/hind legs	Liquid faeces that sifted through the bedding	> 39.4

Only natural occurring disease was recorded in this study. When the research team detected disease that had reached predefined limits, they alerted the farm team. These limits were low milk consumption (< 2 litres by 10:30 am), a depressed demeanour, fever, WCHS > 4 or dehydration. Calves would then be treated according to protocols previously agreed with the farm’s veterinary surgeon.

5.3.3 Behaviour data collection

Activity data was recorded using a triaxial accelerometer attached to each calf’s right hind leg (*IceQube, IceRobotics, Edinburgh*). The data output from the accelerometers included time spent lying and standing, transitions between lying and standing and the motion index for each fifteen-minute interval. Motion index is a proprietary measure of activity. The data was processed to produce daily lying time (minutes), daily standing time (minutes), daily lying bouts (n), daily standing bouts (n), total daily motion index, mean motion index per standing bout, mean lying bout length (minutes), and mean standing bout length (minutes).

The automatic milk feeder recorded the time of each visit where milk was consumed as well as the duration of the visit, milk consumed during the visit, and the speed at which the milk was consumed. From this data, the total time at milk

feeder (minutes), total milk visits (n), mean milk visit length (minutes), mean milk drinking speed (g/s), volume of milk drunk/day (ml) and mean milk per visit (ml) were calculated for each calf.

5.3.4 Data handling and analysis

All data was initially exported into Microsoft Excel (*Microsoft Corporation, Redmond, Washington*). The day of entry into the group pen was removed for each calf to ensure only complete days were included in the analysis. One calf was removed from the data set due to mortality from a condition other than NCD at 9 days of age. Days where there were any problems with the automatic milk feeder and the subsequent day were removed entirely as this was likely to have significant effects on calf behaviour (98 calf days). Reasons for removal included: water supply issues (9 calf days), computer issues (54 calf days), frozen water supply (19 calf days), not mixing properly (13 days) and a calf stuck in the feeder blocking access for others (3 calf days). A further 28 calf days were removed as calves had consumed more than their total allowance including the maximum carry over, possibly due to a computer error or calves being placed on the wrong feeding programme. Incomplete or missing days of activity data (147 calf days) were removed from the activity dataset only. Reasons for removal included: the IceQube having been placed upside down (12 calf days), error in data flow meaning that data had been recorded twice (3 calf days), calves not going close enough to the receiver or IceQube malfunction (57 calf days) and the server/receiver in the shed malfunctioning (75 calf days). As this study is to examine the effect of NCD on calf behaviour, days where WCHS was > 4 were removed to avoid any confounding effect of bovine respiratory disease (milk data: 38 calf days, activity data: 36 calf days).

Data processing and analysis was done using R (R Core Team, 2021) through the R Studio graphical interface (*R studio, Boston, Massachusetts*). Data processing was carried out using the tidyverse package (Wickham *et al.*, 2019) and descriptive statistics calculated using the psych package (Revelle, 2021). Days in September, October and November were classified as in autumn, days in December, January, and February as in winter and days in March and April as in spring.

General linear mixed models were constructed using the lme4 package (Bates *et al.*, 2015). Forward model selection was conducted using the step() function in the lmerTest package (Kuznetsova *et al.*, 2017). The fixed effects included in the maximal model were disease status, season, sex, sire breed type, temperature score, age and the interactions between disease status and age, disease status and season, and disease status and temperature score. The random effect was calf identity nested within group. The residuals were visualised using the simulateResiduals() and plot() functions in the DHARMA package (Hartig, 2022). Where residuals were not normally distributed, a transformation was used. A square root transformation was used for daily lying and standing time, mean lying and standing bout length, total daily motion index, mean motion index per standing bout and mean milk visit length. A natural log transformation was used for daily lying and standing bouts. A square transformation was used for mean milk drinking speed. Estimates, t values, and p values were calculated using the summary() function in the lmerTest package (Kuznetsova *et al.*, 2017). The confidence intervals of the estimates were calculated using the confint() function (Bates *et al.*, 2015) and the numerator degrees of freedom and denominator degrees of freedom were calculated using the anova() function (R Core Team, 2021). To visualise the results, estimated marginal means (EMM), their standard errors and pairwise comparisons were calculated using the emmeans() function in the emmeans package (Lenth, 2024). These were then plotted using ggplot2 (Wickham, 2016).

As the calves were limit fed, the daily milk volume data had a severe right skew. Thus, this data was transformed into a binary variable of whether a calf had drunk its allowance or not each day. This was defined as having drunk ≥ 6500 ml. This was then analysed using a binomial generalised linear mixed model with the glmer() function in the lme4 package (Bates *et al.*, 2015). Each fixed effect was tested in turn and those with $p > 0.2$ were taken forward for forward model building. The fixed effects tested were disease status, season, sex, sire breed type, temperature score, and age. All models contained the random effect of calf identity nested within group. Z values and p values were calculated using the summary() function in the lmerTest package (Kuznetsova *et al.*, 2017). Odds ratios and confidence

intervals were calculated using the `vcov()` and `fixef()` functions in the `lmerTest` package (Kuznetsova *et al.*, 2017) and base R (R Core Team, 2021). The multivariable models that converged were tabulated using `aictab()` (`AICcmodavg` (Mazerolle, 2020)), and the model with the Akaike Information Criterion corrected for small sample sizes (AICc) closest to 0 selected. The model was checked for over dispersion using the equation suggested by Bolker (2021). The raw data was visualised using `ggplot` (Wickham, 2016).

Similarly, due to the way meal allocation worked on the automatic milk feeder the distribution of mean milk per feed was not normal. A linear mixed model with a range of transformations was attempted as described above, but checking of residuals revealed a lack of uniformity and quantile deviations. Thus, the data was split into quartiles representing different mean daily meal sizes (very small (0 – 702 ml), small (703 – 913 ml), medium (914 – 1129 ml) and large (1130 – 1400 ml)). This data was then analysed using a cumulative link model by using the `clmm()` function in the `ordinal` package (Christensen, 2023). Forward model building was carried out by testing each fixed effect in turn and those with $p < 0.2$ were taken forward for model building.

The fixed effects tested were disease status, season, sex, sire breed type, temperature score, age and the interactions between disease status and age, disease status and season, and disease status and temperature score. The calf identity nested within its group was included in all models as a random effect. Z values and p values were calculated using the `summary()` function. The final model was selected using the AICc with the models tabulated using `aictab()` (`AICcmodavg` (Mazerolle, 2020)). The model with the AICc closest to 0 was selected. The odds ratios were calculated using base R (R Core Team, 2021). The raw data was visualised using `ggplot` (Wickham, 2016). Probabilities of each meal size and the standard errors were calculated using the `emmip()` function in the `emmeans` package (Lenth, 2024) and visualised using `ggplot()` (Wickham, 2016).

5.4 Results

Of the 141 calves in the study, 108 calves were diagnosed with diarrhoea. Ninety-eight of these did not develop dehydration, while ten calves developed dehydration for one day only. Once data cleaning was completed for the activity variables, there were 135 calves with 933 healthy days, 239 NCD-H days and 7 NCD-D days between them. For the milk feeding variables there were 139 calves with 1044 healthy days, 270 NCD-H days and 9 NCD-D days between them. The distribution of calves and calf days across diseases categories, sex, season, and disease type are seen in Table 5.3 for the milk feeding data and Table 5.4 for the activity feeding data. Descriptive statistics for each milk feeding and activity variable are seen in Table 5.5.

Table 5.3 The number of calf days and corresponding calves in the milk feeding data broken down by sex, sire-breed-type and season.

Sex	Season	Sire breed type	Healthy		NCD-H		NCD-D	
			Calf days	Number of calves	Calf days	Number of calves	Calf days	Number of calves
Female	Autumn	Beef	196	27	47	20	3	3
		Dairy	43	9	20	8		
	Winter	Beef	97	13	17	8		
		Dairy	45	8	18	7	2	2
	Spring	Beef	166	17	37	14		
		Dairy	17	1				
Male	Autumn	Beef	170	23	42	16	3	3
		Dairy	5	1	5	2		
	Winter	Beef	148	18	27	11	1	1
		Dairy	12	2	6	2		
	Spring	Beef	123	17	42	16		
		Dairy	22	3	9	3		

N.B. Calves may appear in multiple disease categories or seasons.

Table 5.4 The number of calf days and corresponding calves in the activity data broken down by sex, sire-breed-type and season.

Sex	Season	Sire breed type	Healthy		NCD-H		NCD-D	
			Calf days	Number of calves	Calf days	Number of calves	Calf days	Number of calves
Female	Autumn	Beef	27	192	19	43	3	3
		Dairy	9	42	8	19		
	Winter	Beef	13	91	8	17		
		Dairy	8	36	7	17	1	1
	Spring	Beef	16	135	12	28		
		Dairy	1	17				
Male	Autumn	Beef	23	160	15	39	2	2
		Dairy	1	5	2	5		
	Winter	Beef	18	137	11	27	1	1
		Dairy	2	12	2	5		
	Spring	Beef	15	95	13	31		
		Dairy	1	11	3	8		

N.B. Calves may appear in multiple disease categories or seasons.

Table 5.5 Descriptive statistics of activity and milk feeding variables in calves 7 - 25 days of age

	Mean	SD	Minimum	Maximum	SEM
Daily lying time (minutes)	1073.2	95.2	374.4	1371	2.8
Daily standing time (minutes)	366.9	95.2	69.0	1065.6	2.8
Daily lying bouts (n)	19.3	5.4	7.0	40.0	0.2
Daily standing bouts (n)	19.3	5.4	6.0	39.0	0.2
Total daily motion index	3490.5	1906.4	247.0	19918.0	55.5
Mean lying bout length (minutes)	60.3	18.6	13.4	195.9	0.5
Mean standing bout length (minutes)	20.4	8.3	3.3	145.0	0.2
Mean motion index per standing bout	185.3	93.7	19.1	766.1	2.7
Total time at milk feeder (minutes)	15.5	5.3	1.9	53.2	0.2
Total milk visits (n)	7.4	2.8	1.0	16.0	0.1
Mean milk visit length (minutes)	2.3	0.9	0.9	8.9	0.0
Mean milk drinking speed (g/s)	7.1	1.9	0.8	15.4	0.1
Daily milk volume (ml)	6199.4	1381.4	1141.0	8431.0	38.0
Mean milk per visit (ml)	908.0	235.3	297.8	1364.4	6.5

After attempting multiple transformations, no model for total time at milk feeder had normally distributed residuals. On reviewing a histogram of the raw data (Figure 5.1) it became clear that this was due to a long right sided tail to the data. As total time at the milk feeder has little biological relevance because it included standing in the feeding station as well as drinking, it was decided to discard this variable.

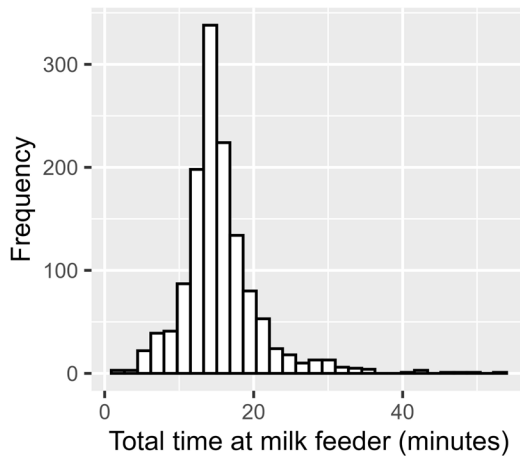


Figure 5.1 Frequency histogram for total time at milk feeder.

Disease status is the main variable of interest in this study therefore the results for the variables where disease status was not involved in any interactions will be presented first. The involvement of disease status in interactions will then be presented and finally the other factors seen in the final models.

5.4.1 The association of neonatal calf diarrhoea with or without dehydration with calf feeding behaviours and activity variables.

5.4.1.1 The associations between disease status and milk feeding behaviours and activity variables.

Disease status was associated with most of the activity variables and the odds ratio of the milk allowance being consumed. Calves with both NCD-H and NCD-D lay down for longer each day than their healthy counterparts ($p = 0.040$ and $p = 0.040$, Table 5.6). However, these differences were no longer present when pairwise comparisons of EMM were carried out ($p = 0.102$, Figure 5.3A). Calves with NCD-H had fewer, longer lying bouts and more standing bouts than their healthy counterparts (daily lying bouts: $p < 0.001$, Figure 5.3B; mean lying bout length: $p < 0.001$, Figure 5.3D; daily standing bouts: $p < 0.001$, Figure 5.3C). Both NCD-H and NCD-D calves were less active in each standing bout when compared to the healthy calves ($p < 0.001$ and $p = 0.020$, Figure 5.3E). Disease status was not associated with either daily standing time or mean standing bout length ($p > 0.05$, Table 5.7). There was no significant difference between NCD-H and NCD-D calves for any of the activity variables ($p > 0.05$, Figure 5.3, Table 5.8). Calves with neonatal calf

diarrhoea were less likely to consume their milk allowance than their healthy counterparts, regardless of hydration status (NCD-H: $p < 0.001$; NCD-D: $p = 0.026$; Table 5.10; Figure 5.2).

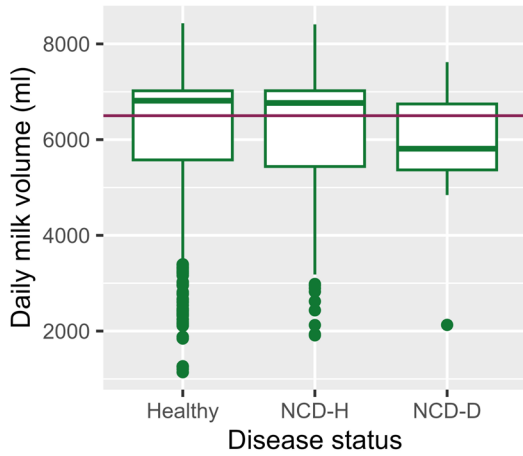


Figure 5.2 A boxplot of the daily milk volume consumed by the three disease categories; the horizontal line indicates the milk volume where it would be considered that a calf had drunk its daily allowance. Healthy: 1044 calf days, 128 calves, NCD-H = Neonatal calf diarrhoea- hydration status normal: 270 calf days, 105 calves, NCD-D = Neonatal calf diarrhoea- dehydrated: 9 calf days, 9 calves.

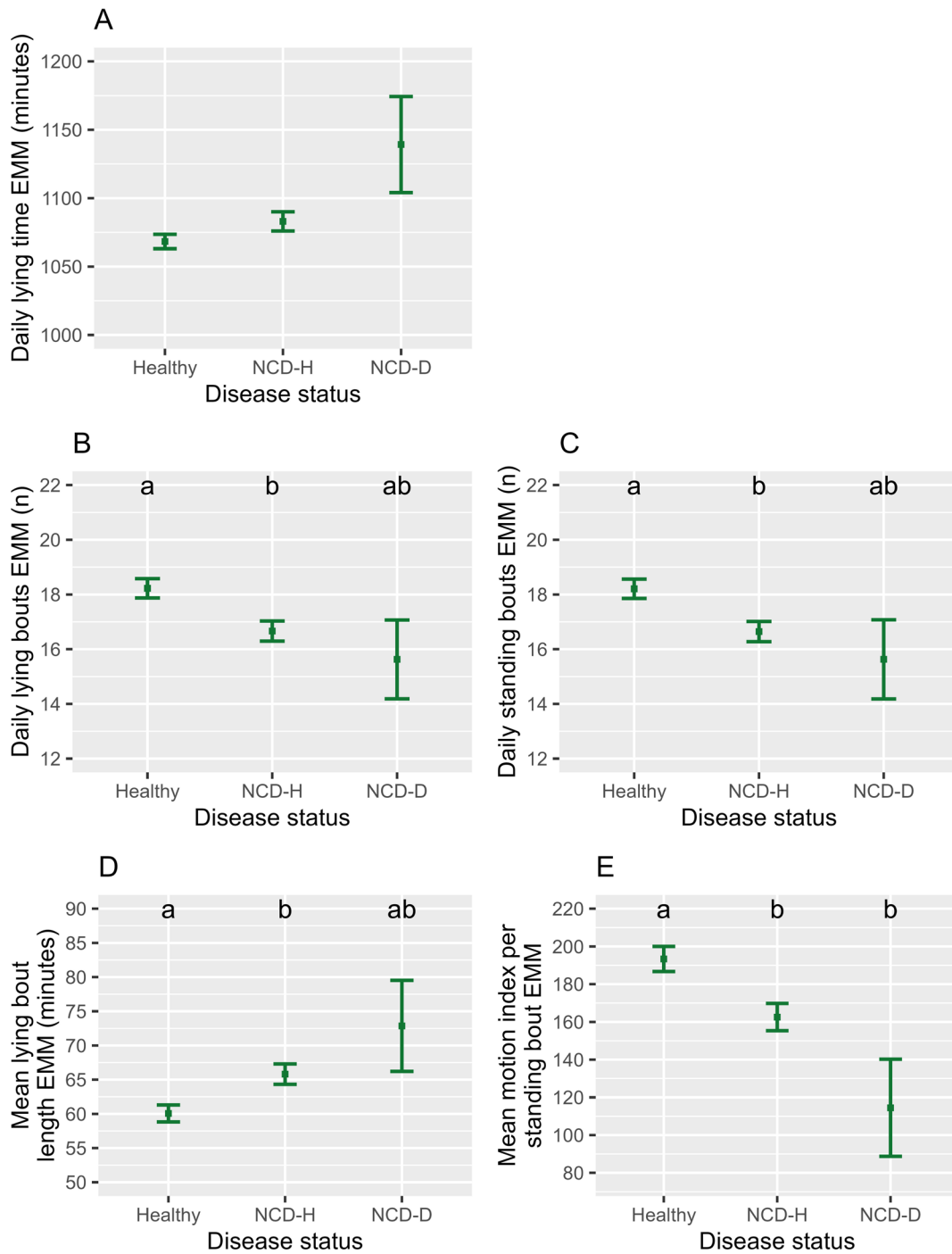


Figure 5.3 The association between disease status and (A) daily lying time, (B) daily lying bouts and (C) daily standing bouts, (D) mean lying bout length, and (E) mean motion index per standing bout as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars denote the standard errors. Differing letters indicate $p < 0.05$. Healthy: 933 calf days, 123 calves, NCD-H = Neonatal calf diarrhoea- hydration status normal: 239 calf days, 98 calves, NCD-D = Neonatal calf diarrhoea- dehydrated: 7 calf days, 7 calves.

5.4.1.2 Interactions between disease status and season for milk feeding behaviours and activity variables

Most of the milk feeding behaviours and the total daily motion index had an interaction between disease status and season in the final model. Generally, diseased calves in autumn were different from their healthy counterparts. However, this effect was seen less commonly in winter and was not seen at all in spring. There were no NCD-D calves in spring (Tables 5.3 and 5.4) and thus no EMM was calculated for NCD-D in spring for any of the variables analysed. In autumn, calves with NCD-H and NCD-D were less active than their healthy counterparts ($p < 0.001$ and $p = 0.003$, Figure 5.4A, Table 5.8), however there was no difference between the two diseased groups and no association of disease with motion index in winter or spring ($p > 0.05$, Figure 5.4A). When pairwise comparisons between seasons for each disease category were performed only one contrast had $p < 0.05$, with healthy calves in winter, being less active than those in autumn ($p < 0.001$, Figure 5.4A).

In the pairwise comparisons for total visits to milk only one comparison had $p < 0.05$. Normally hydrated calves with diarrhoea had significantly fewer visits to milk in autumn than their healthy counterparts ($p < 0.001$, Figure 5.4B). Calves with NCD-H had longer visits to the milk feeder than their healthy counterparts in all three seasons (autumn: $p < 0.001$; winter: $p = 0.007$; spring: $p = 0.029$; Figure 5.4C). In autumn NCD-D calves have longer visits than either healthy or NCD-H calves (Healthy: $p < 0.001$; NCD-H: $p = 0.002$; Figure 5.4C). In spring NCD-H calves visited the milk for a shorter amount of time than in autumn ($p = 0.014$, Figure 5.4C).

Healthy calves drank faster than their NCD-H or NCD-D counterparts in autumn ($p < 0.001$ and $p = 0.010$, Figure 5.4D) however there was no difference between NCD-H and NCD-D calves in autumn ($p = 0.333$, Figure 5.4D). In winter and spring there was no significant difference between the disease groups ($p > 0.05$, Figure 5.4D). Calves with NCD-H drank faster in spring than in autumn ($p = 0.035$, Table 5.9, Figure 5.4D), however no other pairwise comparisons were significant ($P > 0.05$, Table 5.9, Figure 5.4D). However, the small number of NCD-D calves (Table 5.3),

particularly in winter, does mean that caution is required when interpreting these interactions.

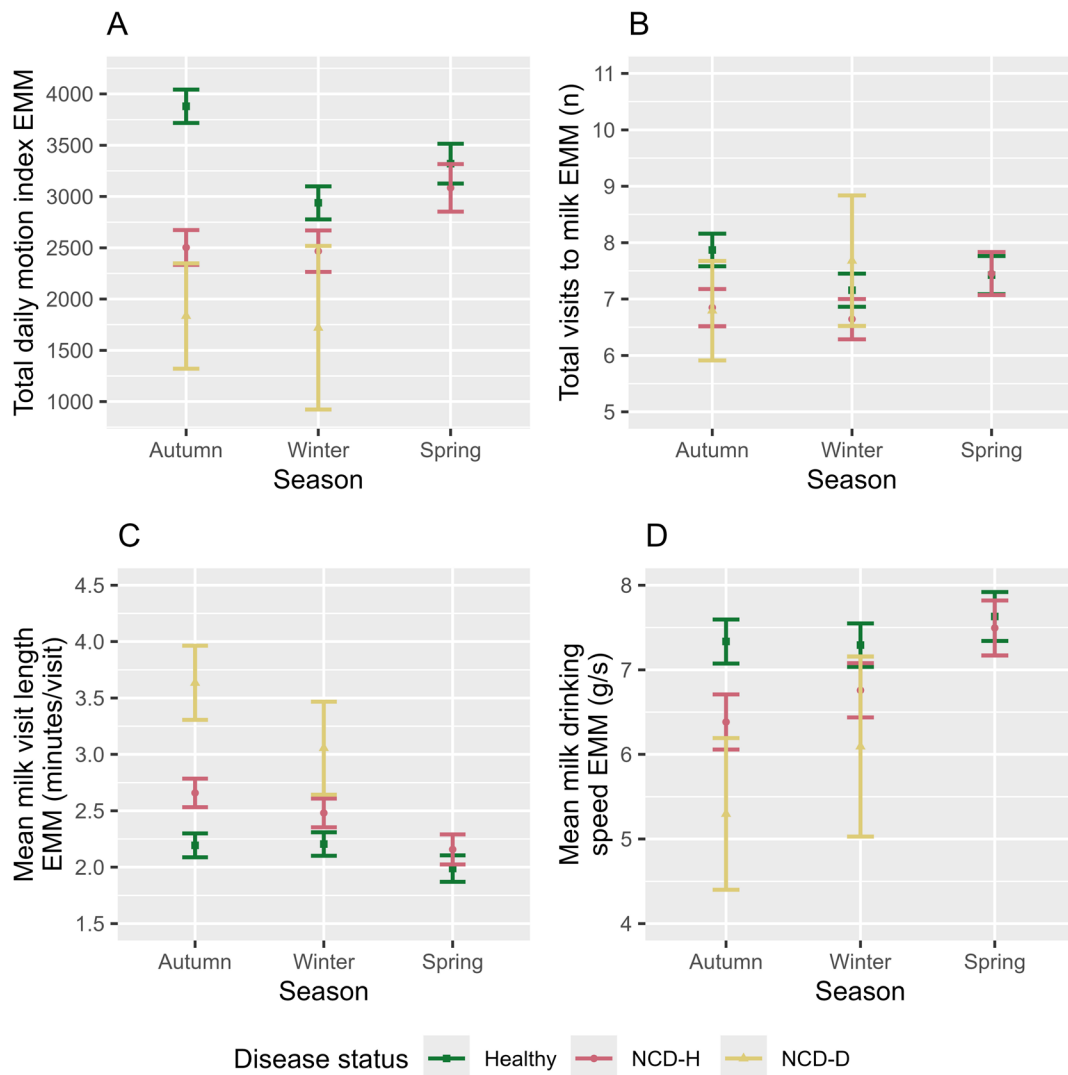


Figure 5.4 The association between disease status and season and (A) total daily motion index, (B) total visits to milk (C) mean milk visit length and (D) mean milk drinking speed as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars denote the standard errors. NCD-H = Neonatal calf diarrhoea- hydration status normal, NCD-D = Neonatal calf diarrhoea-dehydrated. For the number of calf days and corresponding calves in each group see Tables 5.3 and 5.4.

5.4.1.3 Interactions between disease status and age for milk feeding behaviours

An interaction between disease status and age was present for both the total visits to milk ($p < 0.001$, Table 5.9) and the mean daily meal size ($p < 0.001$, Table 5.10). Healthy calves had more visits to milk as they got older, but NCD-H calves did not ($p < 0.001$ and $p = 0.714$, respectively, Figure 5.5A). When pairwise comparisons between healthy and NCD-H calves at different ages were performed, young healthy calves had fewer visits to milk than NCD-H calves while older healthy calves had more visits to milk than NCD-H calves. At 8 days of age healthy calves had fewer visits to milk ($p = 0.032$, Figure 5.5A) than NCD-H calves. At 14 to 24 days of age healthy calves visited the milk more often than NCD-H calves ($p \leq 0.001$, Figure 5.5A). Due to the small number of NCD-D days it was not possible to calculate EMM for daily milk visits across the different ages.

An interaction between disease status and age was also seen in the mean daily meal size. The raw data for age and mean daily meal size classified by disease status is seen in Figure 5.5B. The median age of healthy calves having a high mean daily meal size is lower than those having lower mean daily meal sizes. No difference is seen in the median age of NCD-H calves with different mean daily meal sizes. The median age of NCD-D calves with a high mean daily meal size is older than that of NCD-D calves with a lower mean meal size. The interpretation of the raw data is supported by the probabilities shown in Figure 5.6. It is shown that a high mean daily meal size is more probable in a healthy calf at 8 days of age than in an NCD-D calf and vice versa at 24 days of age. Caution is advised when interpreting the results for NCD-D calves as there were only nine NCD-D calves in the dataset.

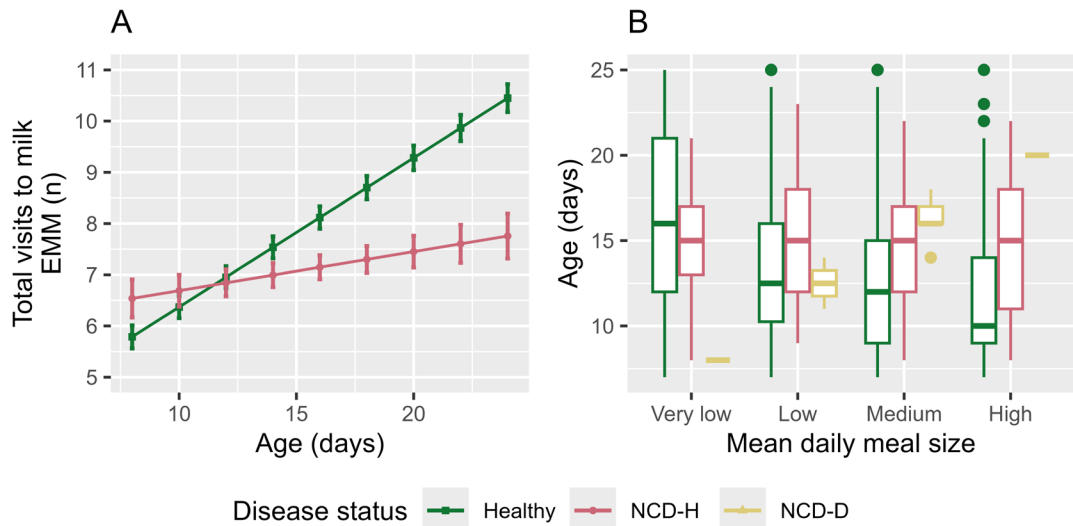


Figure 5.5 (A) The association between disease status and age and total visits to milk as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars denote the standard errors (B) a boxplot showing the age distribution for each mean daily meal size according to disease status. Healthy: 1044 calf days, 128 calves, NCD-H = Neonatal calf diarrhoea- hydration status normal: 270 calf days, 105 calves, NCD-D = Neonatal calf diarrhoea- dehydrated: 9 calf days, 9 calves.

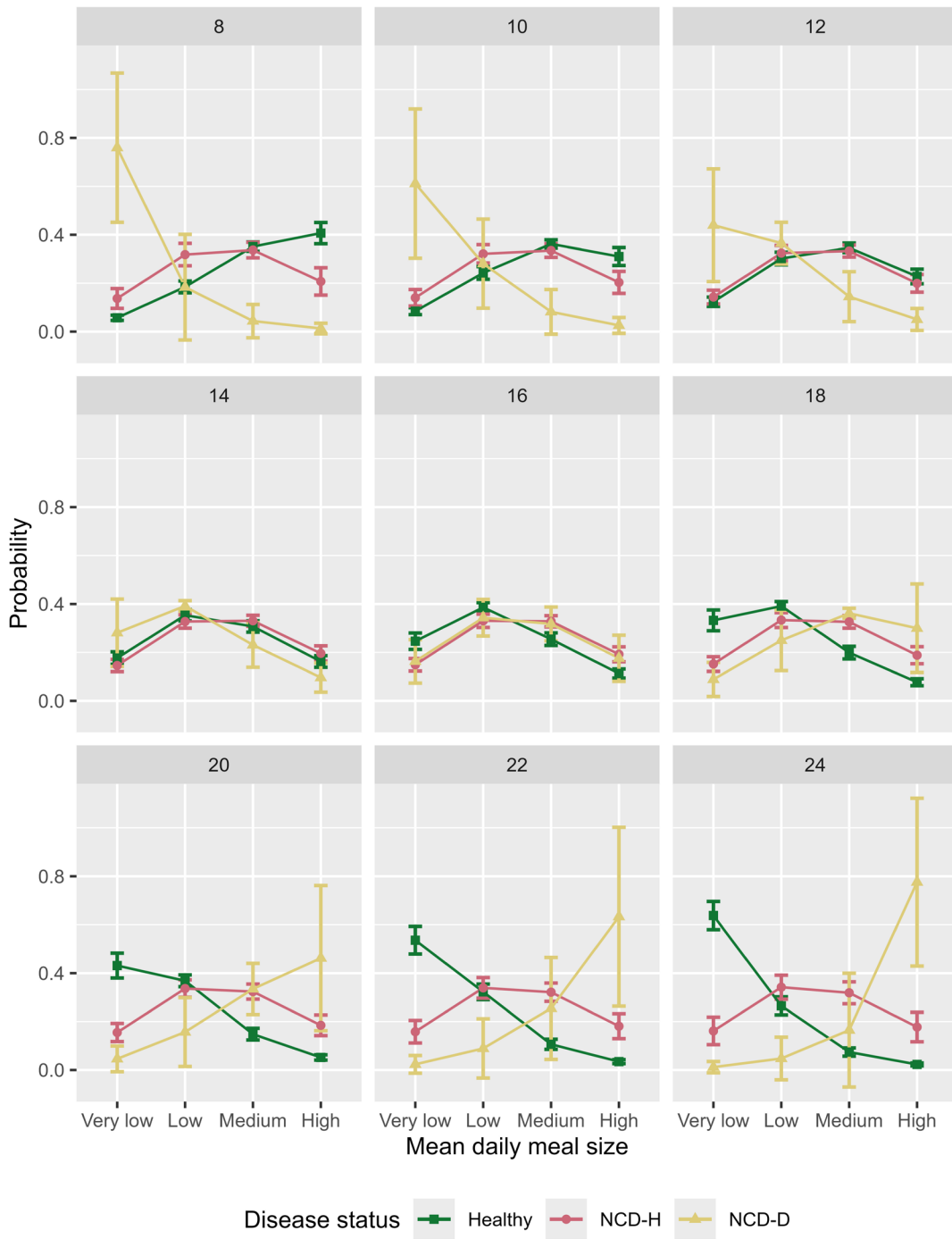


Figure 5.6 Line graphs showing the probability of a calf at different ages having each mean meal size dependent on its disease status. Probabilities are calculated from the final cumulative link mixed model; error bars indicate the standard errors. NCD-H = Neonatal calf diarrhoea- hydration status normal, NCD-D = Neonatal calf diarrhoea- dehydrate

Table 5.6 The effect of neonatal calf diarrhoea with or without dehydration on lying variables of young pre-weaned artificially reared calves. The results shown are from the final linear mixed models^a. Factors with $p < 0.05$ are shown in bold.

Variables	Fixed effect	Level	Number of calf days	Effect size	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t value	P value
Daily lying time (minutes) ^a	Disease status	Healthy	933 ^b	Reference	Reference	2	1124.90	Reference	Reference
		NCD-H	239^c	0.05	-0.00 - 0.19			2.055	0.040
		NCD-D	7^d	1.14	0.00 - 4.34			2.059	0.040
	Age		1179^e	-0.01	-0.01 - -0.00	1	1111.90	-7.966	< 0.001
	Season	Autumn	510 ^f	Reference	Reference	2	213.88	Reference	Reference
		Winter	344^g	0.10	-0.00 - 0.37			2.085	0.038
Spring		325 ^h	-0.05	-0.29 - 0.01	-1.311			0.192	
Daily lying bouts (n) ^a	Disease status	Healthy	933 ^b	Reference	Reference	2	1156.16	Reference	Reference
		NCD-H	239^c	-1.10	-1.13 - -1.05			-4.811	< 0.001
		NCD-D	7 ^d	-1.17	-1.39 - 1.02			-1.692	0.091
	Sire breed type	Beef	1001 ⁱ	Reference	Reference	1	140.91	Reference	Reference
		Dairy	178^j	-1.08	-1.16 - 1.02			-2.446	0.016
	Season	Autumn	510 ^f	Reference	Reference	2	17.27	Reference	Reference
		Winter	344^g	-1.19	-1.27 - 1.12			-5.957	< 0.001
		Spring	325^g	-1.11	-1.18 - -1.04			-3.090	0.009
Mean lying bout length (minutes) ^a	Disease status	Healthy	933 ^b	Reference	Reference	2	1122.62	Reference	Reference
		NCD-H	239^c	0.13	0.04 - 0.42			4.947	< 0.001
		NCD-D	7^d	0.62	0.00 - 2.38			2.096	0.042
	Age		1179^e	-0.00	0.00-0.00	1	1111.44	-3.096	0.038
	Sire breed type	Beef	1001 ⁱ	Reference	Reference	1	141.96	Reference	Reference
		Dairy	178^j	0.10	0.00 - 0.35			2.267	0.021
	Season	Autumn	510 ^f	Reference	Reference	2	13.62	Reference	Reference
		Winter	344^g	0.57	0.062 - 1.01			6.044	< 0.001
Spring		325^h	0.11	0.01 - 0.33	2.333			0.037	

^aThe results shown for the models where a transformation was used have been back transformed.

^b123 calves, ^c98 calves, ^d7 calves, ^e136 calves, ^f62 calves, ^g47 calves, ^h41 calves, ⁱ109 calves ^j26 calves.

Table 5.7 The effect of neonatal calf diarrhoea with or without dehydration on standing variables of young pre-weaned artificially reared calves. The results shown are from the final linear mixed models^a. Factors with $p < 0.05$ are shown in bold.

Variables	Fixed effect	Level	Number of calf days	Effect size	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t value	P value	
Daily standing time (minutes) ^a	Disease status	Healthy	933 ²	Reference	Reference		2	1156.10	Reference	Reference
		NCD-H	239 ³	-0.08	-0.39 - 0.00				-1.658	0.098
		NCD-D	7 ⁴	-2.00	-9.11 - 0.03				-1.734	0.083
	Age		1179⁵	0.02	0.01 - 0.03		1	1134.28	8.648	< 0.001
	Season	Autumn	510 ⁶	Reference	Reference		2	222.91	Reference	Reference
		Winter	344 ⁷	-0.23	-0.95 - 0.00				-1.899	0.059
Spring		325 ⁸	0.22	-0.04 - 1.03				1.686	0.094	
Daily standing bouts (n) ^a	Disease status	Healthy	933 ²	Reference	Reference		2	1213.71	Reference	Reference
		NCD-H	239³	-1.09	-1.143 - -1.05				-4.871	< 0.001
		NCD-D	7 ⁴	-1.17	-1.39 - 1.03				-1.674	0.094
	Sire-breed type	Beef	1001 ⁹	Reference	Reference		1	143.32	Reference	Reference
		Dairy	178¹⁰	-1.09	-1.16 - -1.02				-2.494	0.014
	Season	Autumn	510 ⁶	Reference	Reference		2	19.44	Reference	Reference
		Winter	344⁷	-1.19	-1.27 - -1.12				-5.966	< 0.001
		Spring	325⁸	-1.11	-1.18 - -1.04				-3.018	0.011
	Mean standing bout length (minutes) ^a	Disease status	Healthy	933 ²	Reference	Reference		2	1133.25	Reference
NCD-H			239 ³	0.01	-0.00 - 0.04				1.713	0.087
NCD-D			7 ⁴	-0.00	-0.33 - 0.23				-0.166	0.868
Age			1179⁵	0.00	0.00 - 0.00		1	1195.24	6.935	< 0.001
Sire-breed type		Beef	1001 ⁹	Reference	Reference		1	149.87	Reference	Reference
		Dairy	178¹⁰	0.08	0.01 - 0.24				2.761	0.007
Sex		Female	641 ¹¹	Reference	Reference		1	128.68	Reference	Reference
		Male	538¹²	0.03	0.00 - 0.11				2.164	0.032
Season		Autumn	510 ⁶	Reference	Reference		2	21.03	Reference	Reference
	Winter	344⁷	0.06	0.01 - 0.17				3.045	0.003	
	Spring	325⁸	0.09	0.01 - 0.23				3.352	< 0.001	

¹ The results shown for the models where a transformation was used have been back transformed.

²123 calves, ³98 calves, ⁴7 calves, ⁵136 calves, ⁶62 calves, ⁷47 calves, ⁸42 calves, ⁹109 calves ¹⁰26 calves, ¹¹74 calves, ¹²62 calves.

Table 5.8 The effect of neonatal calf diarrhoea with or without dehydration on activity variables of young pre-weaned artificially reared calves. The results shown are from the final linear mixed models^a. Factors with $p < 0.05$ are shown in bold.

Variables	Fixed effect	Level	Number of calf days	Effect size	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t value	P value	
Total daily motion index ^a	Disease status	Healthy	933 ^b	Reference	Reference		2	1156.10	Reference	Reference
		NCD-H	239^c	-150.19	-228.78 - -87.94				-8.340	< 0.001
		NCD-D	7^d	-378.57	-962.37 - -62.59				-3.294	0.001
	Sex	Female	641 ^e	Reference	Reference		1	117.27	Reference	Reference
		Male	538^f	-17.67	-56.33 - -0.82				-2.477	0.015
	Season	Autumn	510 ^g	Reference	Reference		2	450.12	Reference	Reference
		Winter	344^h	-65.38	-134.65 - -20.62				-4.473	< 0.001
		Spring	325ⁱ	-21.78	-74.26 - -0.49				-2.300	0.022
	Disease status x Season									
Mean motion index per standing bout ^a	Disease status	Healthy	933 ^b	Reference	Reference		2	1156.38	Reference	Reference
		NCD-H	239^c	-1.34	-2.64 - -0.47				-4.822	< 0.001
		NCD-D	7^d	-10.28	-30.79 - -0.78				-2.695	0.007
	Sire-breed type	Beef	1001 ^j	Reference	Reference		1	125.68	Reference	Reference
		Dairy	178^k	1.58	0.15 - 4.36				2.945	0.004

^aThe results shown for the models where a transformation was used have been back transformed.

^b123 calves, ^c98 calves, ^d7 calves, ^e74 calves, ^f61 calves, ^g62 calves, ^h47 calves, ⁱ41 calves, ^j109 calves, ^k26 calves

Table 5.9 The effect of neonatal calf diarrhoea with or without dehydration on milk feeding behaviours of young pre-weaned artificially reared calves. The results shown are from the final linear mixed models^a. Factors with $p < 0.05$ are shown in bold.

Behaviour	Fixed effect	Level	Number of calf days	Effect size	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	t value	P value
Total milk visits (n)	Disease status	Healthy	1044 ^b	Reference	Reference	2	1251.34	Reference	Reference
		NCD-H	270^c	1.95	0.59 – 3.29			2.8727	0.005
		NCD-D	9 ^d	3.90	-2.16 – 9.97		1.254	0.210	
	Age		1323^e	0.29	0.26 – 0.32	1	1301.50	19.733	< 0.001
	Season	Autumn	534 ^f	Reference	Reference	2	96.26	Reference	Reference
		Winter	373^g	-0.71	-1.37 - -0.06			-2.087	0.040
		Spring	416 ^h	-0.45	-1.24 - 0.34			-1.083	0.287
	Age at inclusion into the group pen		1323^e	0.56	0.04 - 1.06	1	128.58	2.177	0.031
		Disease status x Age							< 0.001
		Disease status x Season							0.012
Mean milk visit length (minutes) ^a	Disease status	Healthy	1044 ^b	Reference	Reference	2	1280.19	Reference	Reference
		NCD-H	270^c	0.02	0.01 - 0.04			6.744	< 0.001
		NCD-D	9^d	0.18	0.07 - 0.34			5.344	< 0.001
	Age		1323^e	-0.00	-0.00 - -0.00	1	1300.43	-12.668	< 0.001
	Season	Autumn	534 ^f	Reference	Reference	2	173.05	Reference	Reference
		Winter	373 ^g	0.00	-0.00 - 0.01			0.103	0.918
		Spring	416 ^h	-0.01	-0.03 - 0.00			-1.471	0.147
		Disease status x Season							0.033
Mean milk drinking speed (g/s) ^a	Disease status	Healthy	1044 ^b	Reference	Reference	2	1264.94	Reference	Reference
		NCD-H	270^c	-3.61	-4.22 - -2.88			-5.377	< 0.001
		NCD-D	9^d	-5.07	-6.56 - -2.92			-2.916	0.004
	Age		1323^e	0.91	0.73 - 1.07	1	1285.28	5.324	< 0.001
	Sex	Female	708 ⁱ	Reference	Reference	1	120.98	Reference	Reference
	Male	615^j	2.44	0.96 - 3.33	2.297			0.023	
	Season	Autumn	534 ^e	Reference	Reference	2	170.83	Reference	Reference
		Winter	373 ^f	-0.79	-2.93 – 2.71			-0.152	0.879
		Spring	416 ^g	2.10	-2.37 – 3.80			0.849	0.399
		Disease status x Season							0.027

^aThe results shown for the models where a transformation was used have been back transformed.

^b128 calves, ^c105 calves, ^d9 calves, ^e139 calves, ^f62 calves, ^g47 calves, ^h45 calves, ⁱ75 calves, ^j64 calves

Table 5.10 The effect of neonatal calf diarrhoea with or without dehydration and other factors on the odds ratio of calves having a larger meal size or drinking their daily total milk allowance. Factors with $p < 0.05$ are shown in bold.

Behaviour	Fixed effect	Level	Number of calf days	Odds Ratio	Confidence interval	Z value	P value
Mean daily meal size	Disease status	Healthy	1044 ^a	Reference	Reference	Reference	Reference
		NCD-H	270^b	0.08	0.02 - 0.29	-3.821	< 0.001
		NCD-D	9^c	0.00	0.00 - 0.15	-2.509	0.012
	Age		1323^d	0.81	0.78 - 0.84	-12.514	< 0.001
	Disease status x Age					< 0.001	
Daily milk allowance consumed	Disease status	Healthy	1044 ^a	Reference	Reference	Reference	Reference
		NCD-H	270^b	0.43	0.27 - 0.66	-3.735	< 0.001
		NCD-D	9^c	0.12	0.02 - 0.77	-2.230	< 0.026
	Age		1323^d	1.32	1.25 - 1.39	10.188	< 0.001
	Age at inclusion		1323 ^d	1.45	0.73 - 2.86	1.064	0.287

^a128 calves, ^b105 calves, ^c9 calves, ^d139 calves

5.4.2 The association of other factors with calf milk feeding and activity variables.

A variety of the other factors tested such as season, age and sire-breed type were also present in the final model. These are presented below.

5.4.2.1 The association between season and activity variables

The non-disease factor most seen in the final models was season, which was found in most of the activity variable final models. In spring, calves lay down for less time each day and stood for longer than calves in winter ($p = 0.015$, Table 5.6, Figure 5.7A and $p = 0.008$, Table 5.7, Figure 5.7B). However, neither lying time or standing time differed between autumn and winter or spring (daily lying time: $p = 0.143$ and $p = 0.449$, Table 5.6, Figure 5.7A, daily standing time: $p = 0.191$ and $p = 0.280$, Table 5.7 Figure 5.7B). Calves had fewer lying and standing bouts in winter or spring than they did in autumn ($p < 0.001$ and $p = 0.026$, Table 5.6, Figure 5.7C and $p < 0.001$ and $p = 0.029$, Table 5.7, Figure 5.7D, respectively). However, there was no difference between winter and spring in either daily lying bouts or daily standing bouts ($p = 0.088$, Table 5.6, Figure 5.7C and $p = 0.075$, Table 5.7, Figure 5.7D). Calves had shorter lying bouts in autumn and spring than in winter ($p < 0.001$, and $p = 0.014$, respectively, Table 5.6, Figure 5.7E), but there was no difference between autumn and spring ($p = 0.099$, Table 5.6, Figure 5.7E). In winter and spring, calves had longer standing bouts than in autumn ($p = 0.022$ and $p = 0.020$, respectively, Table 5.7, Figure 5.7F), but there was no difference between winter and spring ($p = 0.847$, Table 5.7, Figure 5.7F).

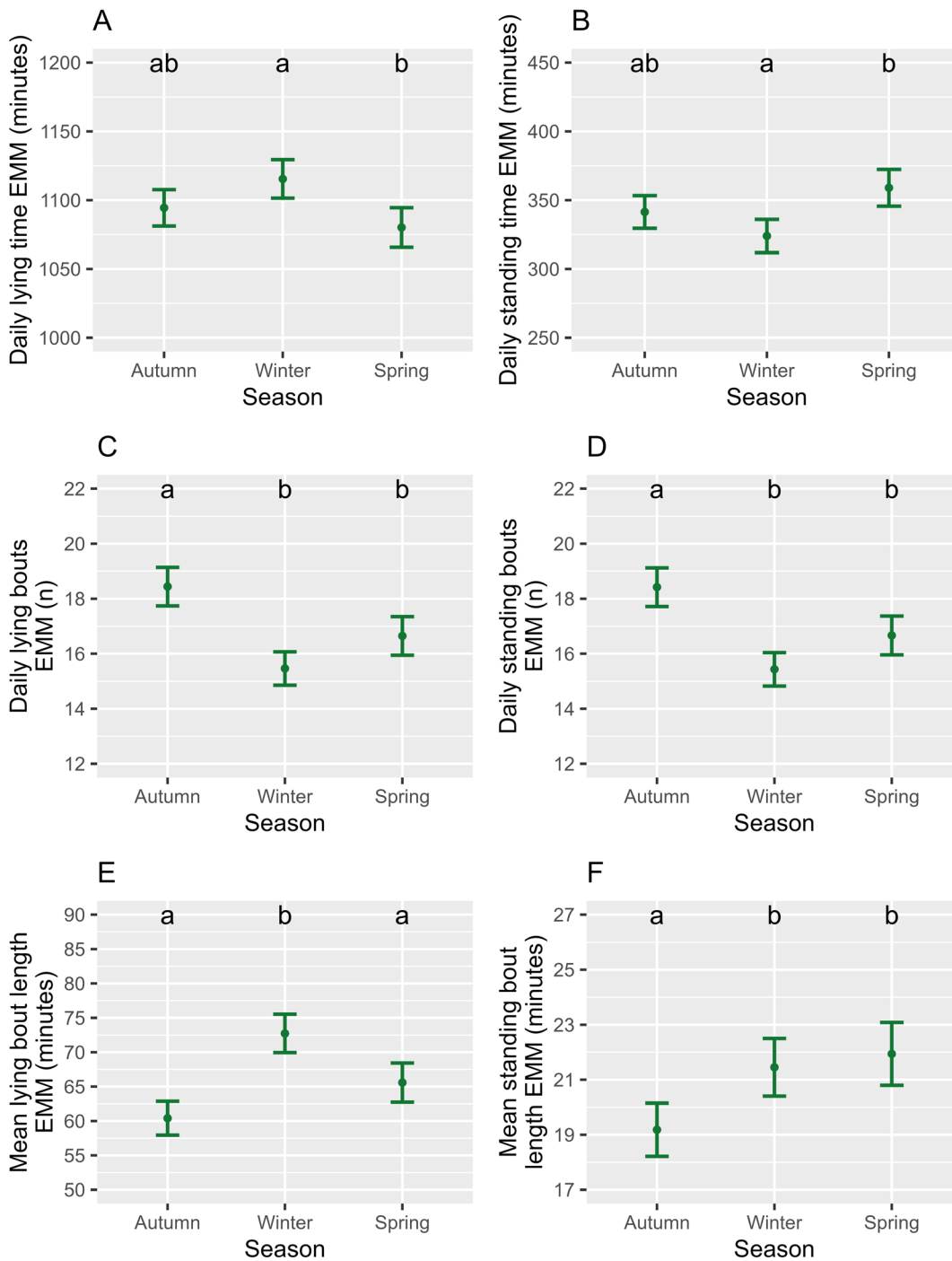


Figure 5.7 The association between season and (A) daily lying time, (B) daily standing time, (C) daily lying bouts, (D) daily standing bouts, (E) mean lying bout length and (F) mean standing bout length as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars denote the standard errors. Differing letters indicate $p < 0.05$. Differing letters indicate $p < 0.05$. Autumn: 510 calf days, 62 calves, Winter: 344 days, 47 calves, Spring: 325 calf days, 41 calves.

5.4.2.2 The association between age and milk feeding behaviours and activity variables.

Age was associated with both milk feeding and activity variables. Older calves lay down for less time each day ($p < 0.001$, Table 5.6, Figure 5.8A) in shorter bouts ($p = 0.038$, Table 5.5, Figure 5.8C). Calves stood for longer ($p < 0.001$, Table 5.7, Figure 5.8B) in longer bouts ($p < 0.001$, Table 5.7, Figure 5.8D) as they got older. Older calves were more likely to drink all of their milk allowance ($p < 0.001$, Table 5.10, Figure 5.9). Age was associated with mean milk drinking speed in the final model ($p < 0.001$, Table 5.9, EMM not estimable).

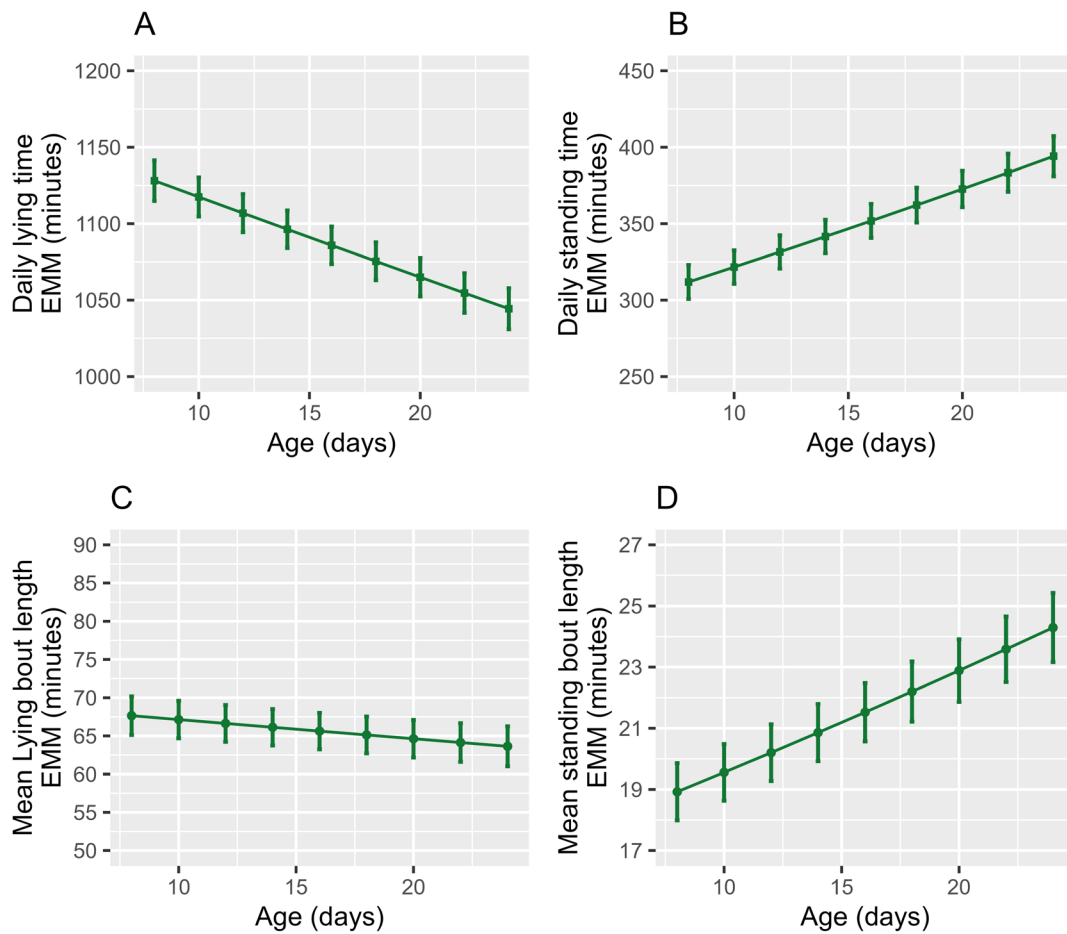


Figure 5.8 The association between age and (A) daily lying time, (B) daily standing time (C) mean lying bout length and (D) mean standing bout length as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars denote the standard errors. Differing letters indicate $p < 0.05$.

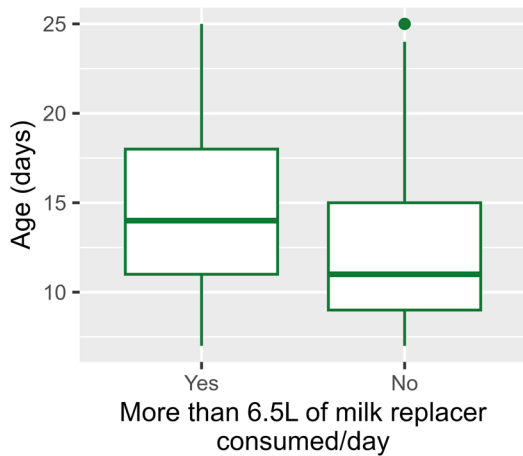


Figure 5.9 A boxplot showing the ages of calves according to whether they had consumed their daily milk allowance or not.

5.4.2.3 The association between sire-breed type and activity variables

Sire-breed type was associated with the characteristics of both lying and standing bouts. Calves with a dairy sire had fewer, longer lying and standing bouts than calves with a beef sire (number of lying bouts: $p = 0.017$, Table 5.6, Figure 5.10A; number of standing bouts: $p = 0.022$, Table 5.7, Figure 5.10B; lying bout length: $p = 0.023$, Table 5.6, Figure 5.10C and standing bout length: $p = 0.007$, Table 5.7, Figure 5.10D). Calves with a dairy sire were also more active per standing bout, ($p = 0.004$, Table 5.8, Figure 5.10E).

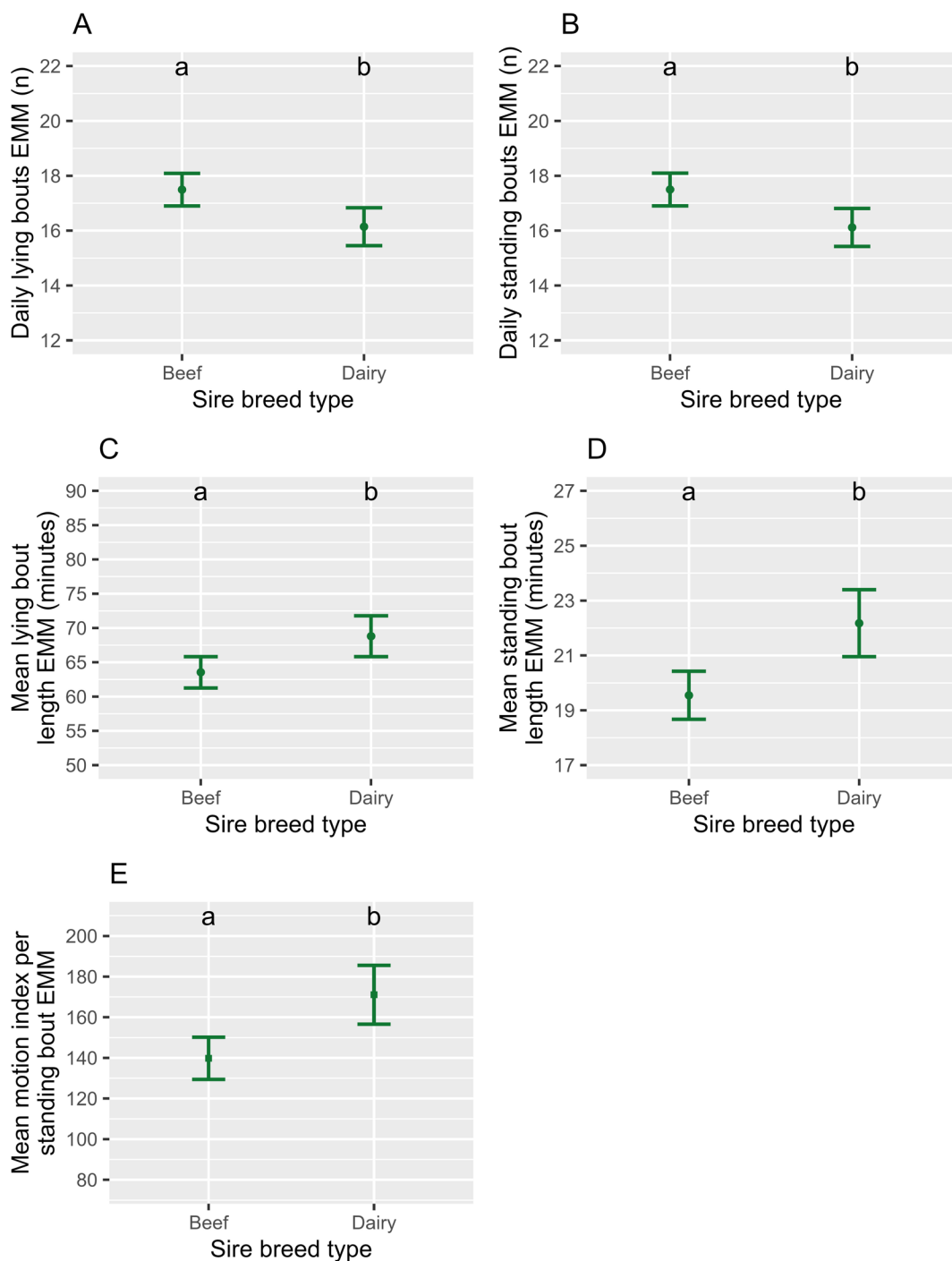


Figure 5.10 The association between sire breed type and (A) daily lying bouts, (B) daily standing bouts, (C) mean lying bout length, (D) mean standing bout length and (E) mean motion index per standing bout as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars denote the standard errors. Differing letters indicate $p < 0.05$. Beef sired calves: 1001 calf days, 109 calves, Dairy sired calves: 178 calf days, 26 calves.

5.4.2.4 The association between sex and milk feeding behaviours and activity variables.

Sex was associated with some of the variables analysed. Male calves had longer standing bouts than their female counterparts ($p = 0.034$, Table 5.7, Figure 5.11). Male calves were less active ($p = 0.001$, Table 5.8, EMM not estimable), and drank faster ($p = 0.023$, Table 5.9, EMM not estimable) than their female counterparts.

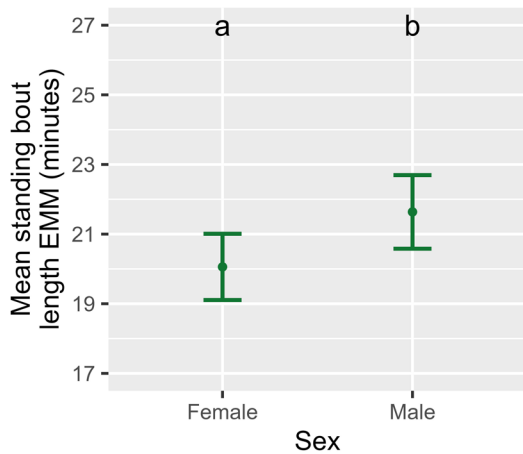


Figure 5.11 The association between sex and daily standing bout length as illustrated by the estimated marginal means (EMM) calculated from the final model. Error bars denote the standard errors. Differing letters indicate $p < 0.05$. Female: 641 calf days, 74 calves, Male: 538 calf days, 61 calves.

5.4.2.5 The association between age at inclusion into the group pen and milk feeding behaviours.

The age at which calves were moved into the group pen was associated with the number of visits to milk. Calves that were moved into the group pen at an older age visited the milk more often ($p = 0.031$, Table 5.9, EMM not estimable). Age at inclusion into the group pen was included in the model for whether a calf had consumed its milk allowance as it improved model fit ($p > 0.05$, Table 5.10).

5.5 Discussion

This study aimed to characterise the association between neonatal calf diarrhoea (NCD) with and without dehydration and lying, standing, activity and milk feeding variables. Regardless of hydration status, calves with NCD were less active in each standing bout and less likely to consume their milk allowance each day than healthy calves. Calves with NCD and a normal hydration status had fewer, longer lying bout and more standing bouts than healthy calves. In autumn, calves with NCD, regardless of hydration status, were less active, had longer visits to milk and drank more slowly than their healthy counterpart. Calves with NCD and dehydration had longer visits to milk than those with NCD and a normal hydration status in autumn. Diarrheic calves that were normally hydrated visited the milk feeder less often in autumn and, visited the milk feeder for longer in all three seasons when compared to their healthy counterparts. Other factors including season, age and sire-breed type were also associated with lying, standing, activity and milk feeding variables.

Previous work in group fed calves using an automatic feeder found that 82 per 100 calves developed diarrhoea in the first sixty days of life, with both a faeces score ≥ 2 and depression (Medrano-Galarza *et al.*, 2018a); 31% of these had severe diarrhoea requiring treatment for > 2 days or intravenous fluids. In this study, the case definition for NCD-H only required a faeces score of ≥ 2 for one day and the incidence was slightly lower at 77 per 100 calves in the first 25 days of life, with 9 per 100 calves developing dehydration in the first 25 days of life. Thus, incidence was lower in the current study, particularly of severe disease. While the incidence of disease was higher in the current study than the previously recorded incidence in the UK of 48 per 100 calves in the first nine weeks of life (Johnson *et al.*, 2017) or 65 per 100 calves in the first six weeks of life (Johnson *et al.*, 2019), it is lower than that found in other studies that monitored for diarrhoea daily (Olson *et al.*, 2019, McCarthy *et al.*, 2024). There was no mortality in this study, likely due to the calves being closely monitored within the study period.

Previous research showed that calves with NCD had fewer, but longer lying bouts and a reduced daily lying time (Lowe *et al.*, 2019). In the current study, calves with NCD and a normal hydration status had fewer longer lying bouts, however daily lying time was increased in calves with NCD, regardless of hydration status, this difference was not seen in pairwise comparisons of the EMM. In contrast, Studds *et al.* (2018) found no difference in the frequency or length of lying bouts or the daily lying time between calves with and without NCD, however in that study, calves were housed in individual pens. In another individual pen study, calves with NCD had a longer daily lying time, the same number of lying bouts and a reduction in activity index than healthy calves (Guevara-Mann *et al.*, 2023). The differences in behaviour seen in these studies may reflect a difference in feeding with calves fed two or three set times a day rather than having free access to the automatic milk feeder. Thus, in individual pens calves may spend more time standing as they are hungry and awaiting their next meal while calves on an automatic milk feeder have the next meal more readily available. All three of the studies above, and the current one, used different bedding materials which may also have influenced their behaviour, particularly the number of lying bouts (Sutherland *et al.*, 2017).

In the current study, only ten calves became dehydrated, with only seven days of activity data and nine days of milk feeding data available between them. These small

numbers compared to the overall number of calf days (1179 and 1323, respectively) is likely to be why only mean milk visit length in autumn showed a significant difference between calves with NCD that were normally hydrated and those that were dehydrated. Further work featuring more calves with moderate to severe disease is required to assess whether activity and milk feeding variables differ between normally hydrated and dehydrated calves with NCD. Further, discussion of the limitations of the disease detection methods in this study are discussed in section 4.7 above.

In this study, calves with NCD with or without dehydration drank more slowly than healthy calves in autumn and were less likely to drink their milk allowance. This is consistent with previous research (Knauer *et al.*, 2017, Lowe *et al.*, 2019, Conboy *et al.*, 2021, Sutherland *et al.*, 2018). However, unlike the previous studies, in this study, calves with NCD that were normally hydrated had fewer visits to milk in autumn. This is consistent with the findings of Conboy *et al.* (2022) who found a reduction in the number of rewarded visits to milk as well as milk intake on the day of disease detection. Sutherland *et al.* (2018) also found that milk consumption declined and there was a decrease in the number of unrewarded visits to the feeder in the days before NCD detection. A limitation of the current study was that the feeders used did not record unrewarded visits. Unrewarded visits may represent sampling behaviour in calves, whereby they are learning about their surroundings (Weary *et al.*, 2009), however they may also be due to hunger (De Paula Vieira *et al.*, 2008, Welk *et al.*, 2023).

The interaction between season and disease in total daily motion index, the number and length of visits to milk and the mean drinking speed is interesting and may reflect a differing response to disease depending on the environmental conditions and needs further exploration, with a greater number of calves. Interpretation of this interaction for dehydrated calves with diarrhoea is limited due to the small numbers (Tables 5.3 and 5.4). This interaction is plausible however as it is known that sickness response may differ according to a variety of external factors (Sylvia and Demas, 2017). Some authors have used season in their disease prediction models. Belaid *et al.* (2020) included season in their model to predict sick calves using daily step count, daily lying time and daily visits to the feed bunk. They achieved best accuracy on the day prior to sickness developing with a sensitivity of 68.8% and specificity is 72.4% (Belaid *et al.*, 2020).

As shown in Chapter 2 (Riley *et al.*, 2023) a variety of calf factors are associated with calf behaviours. This study was conducted over a slightly different period within the year to Chapter 2 (Riley *et al.*, 2023) (September to April rather than July to March). The variable 'season' in this study was defined based on the date on which the behaviour was performed, while in Chapter 2 the date of birth was used. This may in part explain the differences between the findings. The increase in lying bouts seen in autumn may be consistent with the same finding in summer born calves in Chapter 2 (Riley *et al.*, 2023) and previous findings that calves change posture more frequently when they have less access to shade (Kovacs *et al.*, 2018). The increased lying time in winter compared to spring may be due to calves avoiding windy conditions (Bell *et al.*, 2019). An increase in temperature humidity index has been associated with a reduction in drinking speed (Montes *et al.*, 2023). It is possible that this accounts for the reduction in drinking speed in autumn in NCD-H calves.

In contrast to Chapter 2 (Riley *et al.*, 2023) where older calves tended to have a shorter mean standing bout length and were less active, in the current study, standing bouts got longer in older calves and total daily lying time decreased, with an associated increase in daily standing time. The finding in the current study is consistent with that of Hill *et al.* (2013). Previous work showed an increase in lying bouts in 40-day old calves when compared to those at 26 days of age, this was not observed in this study (Jensen *et al.*, 2020). In Chapter 2 (Riley *et al.*, 2023) live-weight was used, however due to the large amount of time involved in cleaning live-weight data for this chapter, it was decided to use the moderately correlated age (Table A1.2, Riley *et al.*, 2023, supplementary material). Birthweight was not included in this study, as this data was missing for seven calves. Older calves had shorter milk visits, were more likely to drink their milk allowance and drank faster, while healthy older calves visited the feeder more often and were more likely to have a low mean daily meal size. This is largely consistent with the findings for live-weight in Chapter 2 (Riley *et al.*, 2023) although mean daily meal size was associated with age in this study and mean milk per feed was not associated with live-weight or age in Chapter 2 (Riley *et al.*, 2023). Graham *et al.* (2024) also found that older calves drank faster. The interactions between age and disease in the models for total visits to milk and mean daily meal size are interesting

and may reflect a differing response to disease as calves age and demonstrate the importance of considering calf factors when building disease detection tools.

There is very limited research looking at the effect of breed on calf behaviour, the majority of which looks at personality types e.g. Hoppe *et al.* (2010). The association between sire breed type and mean lying bout length is consistent with the findings in Chapter 2 (Riley *et al.*, 2023). However, while the previous study found no association between the number of lying and standing bouts, the length of standing bouts or the activity per standing bout and sire breed type, it is possible that this is due to the larger amount of activity data available in this study (303 calf days (31 calves) vs 1104 calf days (104 calves)). Unlike in Chapter 2 (Riley *et al.*, 2023), there was no effect of sire breed type on mean milk drinking speed in this study. This is consistent with the findings of Arens *et al.* (2023) that found no difference in drinking speed between Holstein, dairy crossbred, and dairy crossbred cross Limousin calves. However, Limousin crossed calves consumed less milk than the Holstein or dairy crossbred calves, this effect may not have been seen in this study due to the limit on milk intake.

Male calves drank faster than their female counterparts, this is the opposite of that found in Chapter 2 (Riley *et al.*, 2023) where male calves drank slower than their female counterparts. Ugwu *et al.* (2021) found that female calves walked for longer than their male counterparts which is consistent with the lower total daily motion index seen in male calves in this study compared to the females. Mean standing bout length was longer in male calves in this study, which is a novel finding.

Interestingly intrinsic factors may make calves less able to respond to the challenge of disease. Cantor *et al.* (2022) found that calves that had BRD ten to fourteen days after an antimicrobial treatment had fewer unrewarded visits and lower motion index in the ten days following initial treatment than those that were not showing signs of BRD at ten to fourteen days following treatment. This raises an interesting question as to whether there are intrinsic factors affecting how the relapsed calves responded to disease. These could include the calf's personality as more fearful calves had a greater change in both milk intake and drinking speed following a diagnosis of diarrhoea than less fearful calves (Woodrum Setser *et al.*, 2024). Neave *et al.* (2018) found that calves classified as "interactive" or "vocal-inactive" had more unrewarded visits when step-down weaning commenced than those classified as "exploratory/active".

This study design simply classified calves as diseased or healthy on each eligible day. Other studies have included the day relative to disease detection as part of the study design. This has allowed the exploration of when behavioural changes occur relative to the detection of disease. Interestingly, Swartz *et al.* (2020) found changes in lying behaviours from six to three days prior to disease diagnosis but not immediately prior to or after disease diagnosis. A disadvantage of this approach however is the need for either data for a substantial period prior to disease development to allow a calf to be compared to itself e.g. Knauer *et al.* (2017), or another healthy calf to function as a control e.g. Guevara-Mann *et al.* (2023). Both methods would have been challenging given the amount of disease in this study, the age at which disease developed (mean: 14 days) and the nature of the population (i.e. male and female calves that were both beef and dairy sired).

A significant challenge in the development of disease detection tools based on behaviour is the level of data loss. In this study, 8.5% of the calf days for the milk feeder data and 12.1% of the calf days of activity data were missing or incomplete. Other authors have also encountered this challenge. Knauer *et al.* (2017) excluded 153 calves that had more than ten days of missing data from the milk feeder (12.7%). Goharshahi *et al.* (2021) excluded five calves due to data loss from the ear tag-based accelerometer used in that study (1.6%). Missing data will impact the accuracy of any algorithm used to predict disease and may cause producers to distrust a system. This author supports the suggestions of Tuyttens *et al.* (2022) that PLF systems should be complimented with alarms to signal any mal-functioning technologies and this should include clear ways of identifying unseen sensors.

5.6 Conclusion

In conclusion, NCD with or without dehydration is associated with changes in milk feeding and lying, standing and activity variables. However, further research is needed with more dehydrated calves to ascertain whether differentiation between different levels of disease severity is possible. The use of these variables shows some promise for use in future disease detection tools. However, other calf variables need to be considered, and the challenge of data loss needs to be overcome.

5.7 Bibliography

- ABUELO, A., CULLENS, F. & BRESTER, J. L. 2021. Effect of preweaning disease on the reproductive performance and first-lactation milk production of heifers in a large dairy herd. *Journal of Dairy Science*, 104, 7008-7017.
- ABUELO, A., HAVRLANT, P., WOOD, N. & HERNANDEZ-JOVER, M. 2019. An investigation of dairy calf management practices, colostrum quality, failure of transfer of passive immunity, and occurrence of enteropathogens among Australian dairy farms. *Journal of Dairy Science*, 102, 8352-8366.
- ARENS, S. C., SHARPE, K. T., SCHUTZ, M. M. & HEINS, B. J. 2023. Response to ad libitum milk allowance by crossbred dairy and dairy–beef calves in an automated feeding system. *Translational Animal Science*, 7, txad063.
- BATES, D., MÄCHLER, M., BOLKER, B. & WALKER, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67, 1-48.
- BELAID, M. A., RODRIGUEZ-PRADO, M., RODRIGUEZ-PRADO, D. V., CHEVAUX, E. & CALSAMIGLIA, S. 2020. Using behavior as an early predictor of sickness in veal calves. *Journal of Dairy Science*, 103, 1874-1883.
- BELL, D. J., HASKELL, M. J., MACRAE, A. I., MITCHELL, M. A., JENNINGS, A. & MASON, C. S. 2019. Behavioural responses of dairy calves to temperature and wind. 53rd Annual Congress of the ISAE, 2019. Wageningen Academic Publishers.
- BERCHTOLD, J. 2009. Treatment of Calf Diarrhea: Intravenous Fluid Therapy. *Veterinary Clinics of North America: Food Animal Practice*, 25, 73-99.
- BROWN, A. J., SCOLEY, G., O'CONNELL, N., ROBERTSON, J., BROWNE, A. & MORRISON, S. 2021. Pre-Weaned Calf Rearing on Northern Irish Dairy Farms: Part 1. A Description of Calf Management and Housing Design. *Animals* [Online], 11.
- BOCCARDO, A., SALA, G., COPPOLETTA, E., BRONZO, V., PROSERPIO, M., BELLOLI, A. G. & PRAVETTONI, D. 2019. Frequency and severity of neonatal calf diarrhea cases treated with a standard veterinary hospital protocol do not affect heifer reproduction performance and first lactation production. *Livestock Science*, 230, 103836.
- BOLKER, B. 2021. *GLMM FAQ* [Online]. <https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#overdispersion>: Ben Bolker. [Accessed 21/12/2021 2021].
- CANTOR, M. C., RENAUD, D. L., NEAVE, H. W. & COSTA, J. H. C. 2022. Feeding behavior and activity levels are associated with recovery status in dairy calves treated with antimicrobials for Bovine Respiratory Disease. *Scientific Reports*, 12, 4854.
- CHRISTENSEN, R. H. B. 2023. *ordinal—Regression Models for Ordinal Data*.
- CONBOY, M., WINDER, C. B., CANTOR, M. C., COSTA, J. H. C., STEELE, M. A., MEDRANO-GALARZA, C., VON KONIGSLOW, T. E., KERR, A. & RENAUD, D. L. 2022. Associations

between Feeding Behaviors Collected from an Automated Milk Feeder and Neonatal Calf Diarrhea in Group Housed Dairy Calves: A Case-Control Study. *Animals*, 12, 170.

CONBOY, M. H., WINDER, C. B., MEDRANO-GALARZA, C., LEBLANC, S. J., HALEY, D. B., COSTA, J. H. C., STEELE, M. A. & RENAUD, D. L. 2021. Associations between feeding behaviors collected from an automated milk feeder and disease in group-housed dairy calves in Ontario: A cross-sectional study. *Journal of Dairy Science*, 104, 10183-10193.

DACHRODT, L., ARNDT, H., BARTEL, A., KELLERMANN, L. M., TAUTENHAHN, A., VOLKMANN, M., BIRNSTIEL, K., DO DUC, P., HENTZSCH, A., JENSEN, K. C., KLAWITTER, M., PAUL, P., STOLL, A., WOUDESTRA, S., ZUZ, P., KNUBBEN, G., METZNER, M., MULLER, K. E., MERLE, R. & HOEDEMAKER, M. 2021. Prevalence of disorders in preweaned dairy calves from 731 dairies in Germany: A cross-sectional study. *Journal of Dairy Science*, 104, 9037-9051.

DE PAULA VIEIRA, A., GUESDON, V., DE PASSILLÉ, A. M., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2008. Behavioural indicators of hunger in dairy calves. *Applied Animal Behaviour Science*, 109, 180-189.

GOHARSHAH, M., AZIZADEH, M., LIDAUER, L., STEININGER, A., KICKINGER, F., OHLSCHUSTER, M., AUER, W., KLEIN-JOBSTL, D., DRILLICH, M. & IWERSEN, M. 2021. Monitoring selected behaviors of calves by use of an ear-attached accelerometer for detecting early indicators of diarrhea. *Journal of Dairy Science*, 104, 6013-6019.

GRAHAM, J. R., MONTES, M. E., PEDROSA, V. B., DOUCETTE, J., TAGHIPOOR, M., ARAUJO, A. C., GLORIA, L. S., BOERMAN, J. P. & BRITO, L. F. 2024. Genetic parameters for calf feeding traits derived from automated milk feeding machines and number of bovine respiratory disease treatments in North American Holstein calves. *Journal of Dairy Science*, 107, 2175-2193.

GUEVARA-MANN, D., RENAUD, D. L. & CANTOR, M. C. 2023. Activity behaviors and relative changes in activity patterns recorded by precision technology were associated with diarrhea status in individually housed calves. *Journal of Dairy Science*, 106, 9366-9376.

HARTIG, F. 2022. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. <https://CRAN.R-project.org/package=DHARMA>.

HILL, T. M., BATEMAN, H. G., 2ND, ALDRICH, J. M., QUIGLEY, J. D. & SCHLOTTERBECK, R. L. 2013. Short communication: intensive measurements of standing time of dairy calves housed in individual pens within a naturally ventilated, unheated nursery over different periods of the year. *Journal of Dairy Science*, 96, 1811-4.

HOPPE, S., BRANDT, H. R., KONIG, S., ERHARDT, G. & GAULY, M. 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. *Journal of Animal Science*, 88, 1982-9.

JENSEN, M. B., JENSEN, A. & VESTERGAARD, M. 2020. The effect of milk feeding strategy and restriction of meal patterning on behavior, solid feed intake, and growth

performance of male dairy calves fed via computer-controlled milk feeders. *Journal of Dairy Science*, 103, 8494-8506.

JESSOP, E., LI, L., RENAUD, D. L., VERBRUGGHE, A., MACNICOL, J., GAMSJÄGER, L. & GOMEZ, D. E. 2024. Neonatal Calf Diarrhea and Gastrointestinal Microbiota: Etiologic Agents and Microbiota Manipulation for Treatment and Prevention of Diarrhea. *Veterinary Sciences*, 11, 108.

JOHNSON, K. F., CHANCELLOR, N., BURN, C. C. & WATHES, D. C. 2017. Prospective cohort study to assess rates of contagious disease in pre-weaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. *Veterinary Record Open*, 4, e000226.

JOHNSON, K. F., NAIR, R. V. & WATHES, D. C. 2019. Comparison of the effects of high and low milk-replacer feeding regimens on health and growth of crossbred dairy heifers. *Animal Production Science*, 59, 1648-1659.

KNAUER, W. A., GODDEN, S. M., DIETRICH, A. & JAMES, R. E. 2017. The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, 100, 5642-5652.

KOVACS, L., KEZER, F. L., BAKONY, M., JURKOVICH, V. & SZENCI, O. 2018. Lying down frequency as a discomfort index in heat stressed Holstein bull calves. *Scientific Reports*, 8, 15065.

KUZNETSOVA, A., BROCKHOFF, P. B. & CHRISTENSEN, R. H. B. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1-26.

LENTH, R. V. 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means. <https://CRAN.R-project.org/package=emmeans>.

LOWE, G. L., SUTHERLAND, M. A., WAAS, J. R., SCHAEFER, A. L., COX, N. R. & STEWART, M. 2019. Physiological and behavioral responses as indicators for early disease detection in dairy calves. *Journal of Dairy Science*, 102, 5389-5402.

MAHENDRAN, S. A., WATHES, D. C., BOOTH, R. E. & BLACKIE, N. 2022. A survey of calf management practices and farmer perceptions of calf housing in UK dairy herds. *Journal of Dairy Science*, 105, 409-423.

MAZEROLLE, M. 2020. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). <https://cran.r-project.org/package=AICcmodavg>.

MCCARTHY, H. R., CANTOR, M. C., LOPEZ, A. J., PINEDA, A., NAGORSKE, M., RENAUD, D. L. & STEELE, M. A. 2024. Effects of supplementing colostrum beyond the first day of life on growth and health parameters of preweaning Holstein heifers. *Journal of Dairy Science*, 107, 3280-3291.

MCGUIRK, S. M. 2015. Calf Health Scoring Chart https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf : School of Veterinary Medicine, Wisconsin-Madison University.

- MEDRANO-GALARZA, C., LEBLANC, S. J., DEVRIES, T. J., JONES-BITTON, A., RUSHEN, J., MARIE DE PASSILLÉ, A., ENDRES, M. I. & HALEY, D. B. 2018a. Effect of age of introduction to an automated milk feeder on calf learning and performance and labor requirements. *Journal of Dairy Science*, 101, 9371-9384.
- MEDRANO-GALARZA, C., LEBLANC, S. J., DEVRIES, T. J., JONES-BITTON, A., RUSHEN, J., MARIE DE PASSILLÉ, A. & HALEY, D. B. 2017. A survey of dairy calf management practices among farms using manual and automated milk feeding systems in Canada. *Journal of Dairy Science*, 100, 6872-6884.
- MEDRANO-GALARZA, C., LEBLANC, S. J., JONES-BITTON, A., DEVRIES, T. J., RUSHEN, J., MARIE DE PASSILLE, A., ENDRES, M. I. & HALEY, D. B. 2018b. Associations between management practices and within-pen prevalence of calf diarrhea and respiratory disease on dairy farms using automated milk feeders. *Journal of Dairy Science*, 101, 2293-2308.
- MONTES, M. E., DOUCETTE, J., BRITO, L. F. & BOERMAN, J. P. 2023. Environmental and biological factors that influence feeding behavior of Holstein calves in automated milk feeding systems. *JDS Communications*, 4, 379-384.
- NEAVE, H. W., COSTA, J. H. C., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2018. Personality is associated with feeding behavior and performance in dairy calves. *Journal of Dairy Science*, 101, 7437-7449.
- OLSON, A., SISCHO, W. M., BERGE, A. C. B., ADAMS-PROGAR, A. & MOORE, D. A. 2019. A retrospective cohort study comparing dairy calf treatment decisions by farm personnel with veterinary observations of clinical signs. *Journal of Dairy Science*, 102, 6391-6403.
- PALCZYNSKI, L. J., BLEACH, E. C. L., BRENNAN, M. L. & ROBINSON, P. A. 2021. Stakeholder Perceptions of Disease Management for Dairy Calves: "It's Just Little Things That Make Such a Big Difference". *Animals*, 11, 2829.
- R CORE TEAM 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
- RENAUD, D. L., ROT, C., MARSHALL, J. & STEELE, M. A. 2021. The effect of *Cryptosporidium parvum*, rotavirus, and coronavirus infection on the health and performance of male dairy calves. *Journal of Dairy Science*, 104, 2151-2163.
- REVELLE, W. 2021. psych: Procedures for Psychological, Psychometric, and Personality Research. <https://CRAN.R-project.org/package=psych>. Northwestern University.
- RILEY, B. B., DUTHIE, C.-A., CORBISHLEY, A., MASON, C., BOWEN, J. M., BELL, D. J. & HASKELL, M. J. 2023. Intrinsic calf factors associated with the behavior of healthy pre-weaned group-housed dairy-bred calves. *Frontiers in Veterinary Science*, 10, 1204580.
- RUSSELL, E. R., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2022. Views of Western Canadian dairy producers on calf rearing: An interview-based study. *Journal of Dairy Science*, 105, 1480-1492.

SINNOTT, A. M., KENNEDY, E. & BOKKERS, E. A. M. 2021. The effects of manual and automated milk feeding methods on group-housed calf health, behaviour, growth and labour. *Livestock Science*, 244.

STUDDS, M. J., DEIKUN, L. L., SORTER, D. E., PEMPEK, J. A. & PROUDFOOT, K. L. 2018. Short communication: The effect of diarrhea and navel inflammation on the lying behavior of veal calves. *Journal of Dairy Science*, 101, 11251-11255.

SUTHERLAND, M. A., LOWE, G. L., HUDDART, F. J., WAAS, J. R. & STEWART, M. 2018. Measurement of dairy calf behavior prior to onset of clinical disease and in response to disbudding using automated calf feeders and accelerometers. *Journal of Dairy Science*, 101, 8208-8216.

SUTHERLAND, M. A., WORTH, G. M., CAMERON, C., ROSS, C. M. & RAPP, D. 2017. Health, physiology, and behavior of dairy calves reared on 4 different substrates. *Journal of Dairy Science*, 100, 2148-2156.

SWARTZ, T. H., SCHRAMM, H. H. & PETERSSON-WOLFE, C. S. 2020. Short Communication: Association between neonatal calf diarrhea and lying behaviors. *Veterinary and Animal Science*, 9, 100111.

SYLVIA, K. E. & DEMAS, G. E. 2017. A Return to Wisdom: Using Sickness Behaviors to Integrate Ecological and Translational Research. *Integrative and Comparative Biology*, 57, 1204-1213.

TODD, C. G., MILLMAN, S. T., MCKNIGHT, D. R., DUFFIELD, T. F. & LESLIE, K. E. 2010. Nonsteroidal anti-inflammatory drug therapy for neonatal calf diarrhea complex: Effects on calf performance¹. *Journal of Animal Science*, 88, 2019-2028.

TREFZ, F. M., LORENZ, I., LORCH, A. & CONSTABLE, P. D. 2017. Clinical signs, profound acidemia, hypoglycemia, and hypernatremia are predictive of mortality in 1,400 critically ill neonatal calves with diarrhea. *PLOS ONE*, 12, e0182938.

TUYTTENS, F. A. M., MOLENTO, C. F. M. & BENAÏSSA, S. 2022. Twelve Threats of Precision Livestock Farming (PLF) for Animal Welfare. *Frontiers in Veterinary Science*, Volume 9 - 2022.

UGWU, N., LOVE, E., MURRELL, J., WHAY, H. R., KNOWLES, T. & HOCKENHULL, J. 2021. Development of an ethogram for hutch-housed dairy calves and determination of factors influencing their behaviour. *Applied Animal Behaviour Science*, 234, 105165.

URIE, N. J., LOMBARD, J. E., SHIVLEY, C. B., KOPRAL, C. A., ADAMS, A. E., EARLEYWINE, T. J., OLSON, J. D. & GARRY, F. B. 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. *Journal of Dairy Science*, 101, 9229-9244.

WEARY, D. M., HUZZEY, J. M. & VON KEYSERLINGK, M. A. 2009. Board-invited review: Using behavior to predict and identify ill health in animals. *Journal of Animal Science*, 87, 770-7.

WELK, A., OTTEN, N. D. & JENSEN, M. B. 2023. Invited review: The effect of milk feeding practices on dairy calf behavior, health, and performance: A systematic review. *Journal of Dairy Science*, 106, 5853-5879.

WICKHAM, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*, Cham, Springer-Verlag New York.

WICKHAM, H., AVERICK, M., BRYAN, J., CHANG, W., MCGOWAN, L., FRANÇOIS, R., GROLEMUND, G., HAYES, A., HENRY, L., HESTER, J., KUHN, M., PEDERSEN, T., MILLER, E., BACHE, S., MÜLLER, K., OOMS, J., ROBINSON, D., SEIDEL, D., SPINU, V., TAKAHASHI, K., VAUGHAN, D., WILKE, C., WOO, K. & YUTANI, H. 2019. Welcome to the Tidyverse. *Journal of Open Source Software*, 4, 1686.

WOODRUM SETSER, M. M., NEAVE, H. W. & COSTA, J. H. C. 2024. Are you ready for a challenge? Personality traits influence dairy calves' responses to disease, pain, and nutritional challenges. *Journal of Dairy Science*, 107, 9821-9838.

Chapter 6 General discussion and conclusions

6.0 Preface

The aim of this thesis was to identify possible measures that had potential to be utilised as part of an automated disease detection system in group housed artificially reared pre-weaned limit fed dairy-bred calves. This thesis had five objectives:

- Understand the association between intrinsic calf factors and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves to inform pairing criteria and factors for inclusion in mixed models when analysing the association between these variables and disease.
- Understand the association between bovine respiratory disease and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.
- Understand the association between neonatal calf diarrhoea and saliva conductivity and pH in group housed artificially reared pre-weaned limit fed dairy-bred calves.
- Understand the association between saliva conductivity and pH and physiological indicators of dehydration or acidosis in blood from group housed artificially reared pre-weaned limit fed dairy-bred calves.
- Understand the association between neonatal calf diarrhoea and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.

This discussion will assess whether these objectives have been met. Further opportunities for research will be identified and some of the challenges in this space will be discussed. Finally, there will be a brief discussion of some of the current issues in calf health and welfare and how this thesis relates to them.

6.1 Understand the association between intrinsic calf factors and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves to inform pairing criteria and factors for inclusion in mixed models when analysing the association between these variables and disease.

The first experimental chapter in this thesis sought to address the question of whether there is an association between intrinsic calf factors and calf behaviours. This had interest both for informing pairing criteria and model building for the research in this thesis and as the demographic of artificially reared calves has changed in recent years. Age, live weight, sire-breed type, and sex were identified as being associated with the most behaviours.

However, this work has several important limitations. Firstly, these calves could only consume seven litres of milk a day. Milk allowance has been shown to be associated with several behaviours (see section 1.6.2). This dataset was also small with only twenty-one calves with ten days each included. Due to the messy nature of the live-weight dataset extensive cleaning was required. Therefore, as it is moderately correlated with both age and birthweight (Table A1.2) I would suggest that live-weight could be disregarded in future and age and birthweight used instead. Finally, the dataset was unbalanced for both type and sex. In conclusion while the work in Chapter 2 (Riley *et al.*, 2023) does go some way to addressing this objective more work using a better-balanced dataset, on multiple farms with calves on a variety of nutritional planes is needed to fully understand the associations between intrinsic calf factors and activity and feeding variables.

6.2 Understand the association between bovine respiratory disease and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.

The second experimental chapter in this thesis explored the relationship between milk feeding behaviours and activity variables and BRD using a paired analysis. As discussed in Chapter 3, differences in only three milk feeding behaviours and no activity variables were seen. This may be as the data analysis approach used was a highly controlled

comparison between healthy and diseased animals. This allows the exploration of the development of behavioural changes relative to disease signs reaching their peak. However, by its very nature selecting pairs from the dataset reduces the power of the analysis.

In Chapter 3, calves were defined as having BRD if the WCHS was five or greater.

However, this definition may have a lot of variation within it. Figure 6.1B shows a calf that had WCHS of five on four separate occasions with two to three days of intermediate and normal WCHS in-between each peak, and no administered treatments. In contrast, the calf in Figure 6.1C had one peak of a WCHS of five followed by two intermediate days and then a large peak with a WCHS of ten, when it received a treatment for BRD. This calf went on to have two smaller WCHS peaks however this may be in part influenced by the pharmacodynamics of the treatment administered. It is unlikely that these two calves had the same changes in behaviour, as the second calf is likely to be showing a greater degree of sickness behaviour. Thus, further work looking at associations between disease severity and calf behaviours may be illuminating. The fact that the calf in Figure 6.1B did not receive treatment is consistent with earlier research showing an incongruity between farm and researcher disease detection (Olson *et al.*, 2019).

A further challenge in pairing calves is that very few calves are completely healthy throughout the pre-weaning period. This is illustrated in Figure 6.1A. While this calf could be deemed as healthy in the prescribed period (no treatments and Wisconsin calf health score (WCHS) < 4 in the five days either side of its pairs day of peak disease), it had been treated for both NCD and BRD prior to inclusion in the dataset. However, it would have been incredibly challenging to find pairs that were never diseased, and this would have limited the dataset size further. Of the 108 calves in the dataset, only 13 calves had a WCHS < 4 throughout the pre-weaning period, 23 calves had at least one intermediate WCHS (4) and 72 calves had at least one WCHS consistent with BRD (> 4). This is an incidence of 67 per 100 calves in the pre-weaning period and is consistent with the range of incidence reported by Johnson *et al.* (2017) of between 20 and 78 per 100 calves in the first 9 weeks of life (mean: 46 per 100 calves). Another limitation of this study included an unbalanced dataset (Tables 3.1 and 3.3). This limits the conclusions that can be drawn from the results for live-weight and sex.

It would be advantageous to explore the associations between BRD and activity and milk feeding variables further. This could be done in a variety of ways. The first option would be to use a modelling approach like that used in Chapter 5 on the larger dataset that the Chapter 2 and 3 datasets came from. This would mean that a greater number of calf days were included in the model, however this does not allow the analysis of how behaviours change leading up to disease. If new experimental work were done then several approaches could be taken. Firstly, an inoculation study in which a calf is its own control could be conducted e.g. Hixson *et al.* (2018). Another way would be to run a larger study with many more calves so that more calf pairs can be selected. Finally, data could be collected on a farm where the onset of BRD is later to enable a calf to be its own control.

This research could also be further developed by using the existing data-set to build and test models for predicting disease. There is a variety of modelling approaches that can be taken, these are discussed in section 1.6.4 .

In conclusion, this work partly met the objective but there were several methodological limitations and the effect of a lower milk intake which limits the inferences.

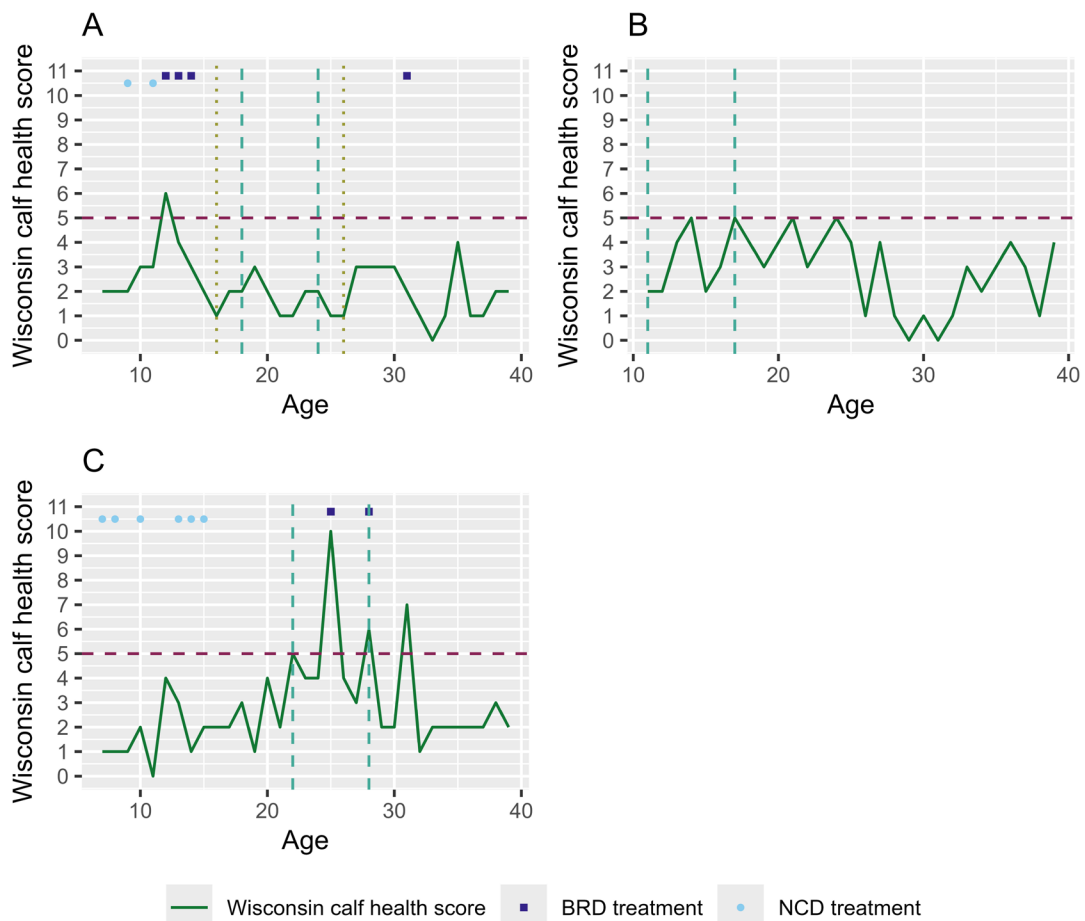


Figure 6.1 The Wisconsin calf health score of three group-housed dairy-bred calves during the pre-weaning period. Treatments for bovine respiratory disease (BRD) and neonatal calf diarrhoea (NCD) are also shown. The vertical dashed lines indicate the period taken for analysis, the vertical dotted lines indicate the period during which a healthy pair had to have received no treatments and have a Wisconsin calf health score < 4. The horizontal dashed line indicates the threshold at which a calf would be considered as having bovine respiratory disease

6.3 Understand the association between neonatal calf diarrhoea with or without dehydration and saliva conductivity and pH in group housed artificially reared pre-weaned limit fed dairy-bred calves.

The fourth chapter of this thesis aimed to address the objective of understanding the association between NCD with or without dehydration and saliva conductivity and pH in calves. The work described in Chapter 4 goes some way to addressing this with diarrhoeic calves having a lowered salivary pH but no difference in saliva conductivity. This study was reasonably well powered for looking at calves with NCD but had insufficient power for looking at dehydration due to a low number of calves becoming severely diseased. This may be due to the calves receiving a preventative treatment for cryptosporidiosis in the first week of life. In the original plan for this trial this treatment

was to be withdrawn, however when this happened during the pilot trial almost all the calves developed severe cryptosporidiosis and became acutely unwell. It is possible that the treatment in the first week of life sufficiently delayed disease until calves had become more resilient. Of the calves that developed NCD during the main trial 59% were positive for cryptosporidium on an immunochromatography tests of the faeces (section 4.3.1).

Since publication of Chapter 4 (Riley *et al.*, 2024b), research developing an electrochemical sensor for glucose and pH detection in calf saliva has been published (Adib *et al.*, 2025). This chip shows some promise, but as yet no device allowing measurement of saliva pH at the teat in the milk feeder has been developed. This would allow daily measurements of the saliva pH for each calf. For pH measurement to be used as part of on farm disease detection, a sensor that can withstand the rigours of daily use would be needed. However, saliva pH on a given day has poor diagnostic accuracy for NCD and moderate accuracy for dehydration (Riley *et al.*, 2024a). Daily changes in saliva pH may prove a more accurate method of detecting diarrhoea or dehydration and further investigation of this is required. A further area of exciting research would be to see if daily saliva pH measurements can be used alongside behavioural data to improve the accuracy of the machine learning models.

Changes in saliva may also be of interest in the detection of BRD. Calves suffering from BRD may develop a respiratory acidosis (Helena *et al.*, 2015). There is some evidence that calves have changes to both the pH and hydrogen peroxide concentration of their exhaled breath concentrate six to twelve days prior to BRD development (Chernitskiy and Safonov, 2021). Further investigation should be conducted to see if this change also presents in saliva or whether gas sampling at the milk feeder is possible. As done to measure methane in adult cattle concentrate feeders e.g. Huhtanen *et al.* (2019).

In conclusion, Chapter 4, addressed the objective of understanding the association between saliva conductivity and pH and NCD with or without dehydration, but inferences for dehydrated calves are limited by low numbers.

6.4 Understand the association between saliva conductivity and pH and physiological indicators of dehydration or acidosis in blood from group housed artificially reared pre-weaned limit fed dairy-bred calves.

Chapter 4 also aimed to address the association between saliva and blood variables. As saliva conductivity was not associated with diarrhoea or dehydration, it was disregarded for this analysis. The relationship between saliva pH and both haematocrit and strong ion difference was negligible. However, the strong ion difference dataset was small, with only thirty samples due to the additional funding required for the electrolyte analysis. Furthermore, a blood gas analysis would have allowed a better assessment of the calf's acid-base status. This was not done in this study as it requires the sample to be analysed within thirty minutes and therefore the costly equipment would need to be on farm for this to be possible. Unmeasured anions have been associated with the demeanour of diarrhoeic calves (Gomez *et al.*, 2013). Therefore, conclusions cannot currently be drawn and further work to explore the physiological mechanism behind the lowered saliva pH in diarrhoeic calves is required.

6.5 Understand the association between neonatal calf diarrhoea and activity and milk feeding variables in group housed artificially reared pre-weaned limit fed dairy-bred calves.

Chapter 5 aimed to address the association between NCD with or without dehydration and activity and milk feeding variables. This objective was partially met and NCD was associated with a reduction in activity and milk being consumed. There were however significant interactions between disease status and both season and age. However, this dataset was not very balanced and in the design of future studies this should be considered. Age will be difficult to balance as when disease occurs will be dependent on the predominant pathogen on that farm.

The dataset used in Chapter 5 was large and messy. Large datasets allow the exploration of the association between disease severity and calf behaviour. The NCD dataset used in Chapter 5 differentiated between dehydrated and normally hydrated calves with NCD. However, the low numbers of dehydrated calves did limit this analysis

and further work on whether differences in behaviour can be used to differentiate between dehydrated and normally hydrated calves with NCD is needed. This may in part be due to the study design where all dehydrated calves were treated as soon as they had been detected, in a commercial setting dehydrated calves may not have been detected so quickly.

In conclusion, this work went some way to understanding the association between NCD and activity and milk feeding variables. However, due to low numbers, inferences on the association between NCD with dehydration and activity and milk feeding variables are limited. Furthermore, given the interactions in the modelling seen with season and age future work should look to balance the dataset across season in particular.

6.6 Which behaviours should we be measuring?

The modelling of milk behaviours, particularly total volume at milk and total milk per feed were constrained in this thesis by these calves being limit feed with access to seven litres of milk a day in a minimum of five feeds at a maximum per feed of 1.4 litres. The total volume of milk per day had to be modelled as a binomial as to whether calves had consumed their daily allowance and in Chapter 5 total milk per feed was modelled as an ordinal. It could be argued that it is not appropriate to look at milk feeding behaviours in calves constrained by the system, however limit feeding is the most common system in the UK with only 6.5% of farms feeding milk *ad libitum* (Mahendran *et al.*, 2022, discussed further in section 6.9 below).

A major limitation of the work in this thesis is that due to the design of the Biocontrol automatic milk feeding system installed at the research farm unrewarded visits to the feeder were not recorded. These are visits to the feeder where the calf has no available milk allowance. Unrewarded visits to the milk feeder are thought by some to be an example of calves expressing exploratory behaviour (Weary *et al.*, 2009) with calves motivated by obtaining information regarding whether milk is available (Wood-Gush and Vestergaard, 1989). However, unrewarded visits may also reflect hunger (De Paula Vieira *et al.*, 2008, Welk *et al.*, 2023). For example, Rosenberger *et al.* (2017) found that unrewarded visits were higher in calves fed six litres of milk compared to those fed twelve litres of milk.

There is considerable evidence that unrewarded visits are reduced in sick calves (Conboy *et al.*, 2021, Svensson and Jensen, 2007, Knauer *et al.*, 2017, Lowe *et al.*, 2019, Morrison *et al.*, 2022). Some studies look at specific calf diseases, calves with NCD had fewer unrewarded visits on the day of and 2 days prior to disease diagnosis (Sutherland *et al.*, 2018). Unrewarded visits are also decreased in calves with BRD (Mahendran *et al.*, 2023, Conboy *et al.*, 2021, Johnston *et al.*, 2016) . However, this was not the case in calves fed a higher level of milk in a study by Cramer *et al.* (2020), or in calves fed *ad libitum* in a study conducted on the same farm as the work in this thesis but using different feeders (Duthie *et al.*, 2021). Surprisingly however while Johnston *et al.* (2016) found a difference in unrewarded visits in the days prior to BRD there was no interaction with plane of nutrition, this may be due to the small size of this study. The improved specificity in the restricted fed calves in the study of Ghaffari *et al.* (2022) may have been driven by the importance of unrewarded visits to the milk feeder in the restricted fed calf model.

A reduction in unrewarded visit may be considered a reduction in a “pliant” or “low-resilience” behaviour. This is a term used for behaviours that are readily re-scheduled in response to varying pressures (Nicol, 2025). These may include exploration, play and grooming. It has been proposed that these behaviours may be more sensitive indicators for disease by in a mouse model for Huntingdon’s disease (Littin *et al.*, 2008) and by Nicol (2025), Stachowicz and Umstatter (2021) and Weary *et al.*, (2009) in their reviews. This is as core behaviours such as lying or rumination may change later in the disease process than pliant behaviours (Stachowicz and Umstatter, 2021).

The use of a cow brush in adult cattle may be another pliant behaviour. Cows with metritis are less likely to use a cow brush and will use the brush for a shorter length of time (Mandel *et al.*, 2017). Pliant behaviours may be involved in trade-offs. Lamé cows are less likely to use a brush further away from the feed than non-lamé cows, however, if that brush is located near the feed then no difference between lamé and non-lamé cows was seen (Mandel *et al.*, 2018). A study in older beef animals suggests that BRD is not associated with brush use (Toaff-Rosenstein and Tucker, 2018).

Play may also be a relevant behaviour, play tends to be carried out when the cost is considered low (Held and Špinka, 2011). Play has been found to be correlated with total acceleration and thus can be measured automatically (Rushen and de Passillé, 2012).

However, play may not be suitable for use as there is significant variation in “playfulness” between individuals (Held and Špinka, 2011).

There are a few considerations for research in this area. Firstly, to use pliant behaviours for disease detection sufficient opportunity to display these behaviours needs to be given e.g. provision of brushes (Stachowicz and Umstatter, 2021). Secondly, it may be more appropriate for research around unrewarded visits to be directed towards preventing hunger in calves (see section 6.9 for discussion on calf diets). Another area of research should be behaviours that may be able to differentiate between disease and distress for other reasons, some behaviours may increase during distress and decrease during disease e.g. grooming (Stachowicz and Umstatter, 2021). In conclusion, further work on pliant behaviours and whether they can be used to increase disease detection accuracy is required.

6.7 Developing disease detection tools that are fit for purpose.

The use of technology on farm provides opportunities to save on labour, detect disease early and could result in higher farm incomes (Dawkins 2025). Particularly as there is a shortage of skilled labour on dairy farms with high staff turnover (Barkema *et al.*, 2015). The increasing use of technology does not come without social and ethical concerns, many of which are discussed in section 1.8 of this thesis. However, there are several other issues that the industry needs to consider including model accuracy, availability of accuracy information and the need for decision support tools and the evidence to back them up.

Overall, the modelling approaches currently available in the literature require some improvement to allow the accurate detection of disease on farm, this is demonstrated by the selection of papers shown in Table 6.1. One of the challenges is that while sickness behaviours are associated with an immune response very similar changes in behaviour may be associated with other conditions e.g. lameness or a metabolic event (Nicol, 2025). This is because sickness behaviours are motivational and non-specific (Cramer and Ollivett, 2020). Therefore, it is important to decide whether the aim is the detection of a specific disease or welfare-related issues in general. Creating more disease specific alerts may also benefit technology adoption (Eckelkamp, 2019).

When looking to improve the models used for disease detection it may be that improving specificity should be the aim as models by Theurer *et al.* (2015) suggested that this would have greater economic gain. Furthermore, if systems are not specific, then there is concern that antibiotics may be used unnecessarily (Buczinski and Pardon, 2020), as there will be a higher number of false positives, particularly if prevalence is low. This is of particular concern when antibiotic resistance has been identified in bacterial BRD pathogens (Melchner *et al.*, 2021). However, if this comes at the cost of a lowered sensitivity this may in fact increase treatments as animals would have more advanced disease by the time it was detected. More advanced disease is also likely to be associated with a greater effect of disease on the calf's welfare. The need for high sensitivity is supported by Van De Gucht *et al.* (2017) who found when farmers were deciding on whether to invest in a lameness detection system how many lame animals a system misses is as important as the number of false alerts.

As discussed above the test characteristics such as the sensitivity and specificity can provide useful information (White *et al.*, 2016). However, they are frequently not reported (Cramer and Ollivett, 2020). Work looking at the behaviour of farmers using a disease detection system in adult cows only checked on an individual cow after 21% of alerts, farmers were more likely to check on cows if they received a lower number of daily alerts further emphasising the need for a high specificity (Eckelkamp and Bewley, 2020). This is partly due to a reduction in trust where false alerts are produced and thus producers prioritizing alerts according to their "gut", available time and work enthusiasm (Dominiak and Kristensen, 2017). The work of Doidge *et al.* (2024) should also be considered when developing models for disease detection, they highlighted that farmers value technology that alerts them to disease early. This raises questions over the systems currently on the market, which do not have any test characteristics published to this author's knowledge. This author feels strongly that technologies on the market should publish the sensitivity and specificity data to allow veterinary surgeons and other advisers to compare systems and be able to give the best advice to their clients. This has been done in other disease detection technologies such as a machine vision lameness detection system for adult cows (Siachos *et al.*, 2025) which was comparable to human mobility scorers. Technologies also need to be externally validated so that producers and their advisors know that they can be trusted, the

review by Stygar *et al.* (2021) suggested that only 14% of systems are externally validated. This can lead to distrust if systems do not work as advertised and effect the uptake of these technologies (Eastwood and Renwick, 2020). There are a variety of market pressures that may lead to the lack of external validation and accuracy information, Eastwood and Renwick (2020) reported that a competitive market and the fact that research and development is frequently privately funded means that there is pressure on companies to get products to market quickly. It has been suggested previously that public organisations may hold a role in setting standards for technological tools (Eastwood *et al.*, 2017). I propose that standards should be set for disease detection tools by the industry and experts and upheld by a public body (e.g. International Standards Organisation) to ensure manufacturers must publish external validation data and obtain a certain level of accuracy prior to going to market. This was suggested previously by Tuytens *et al.* (2022) to reduce the risk of product failure affecting animal welfare.

The combining of different behaviours to predict disease has shown some promise in dairy cow studies (Steensels *et al.*, 2016). A challenge in the implementation of at present producers must frequently look at multiple outputs (Eckelkamp, 2019), possibly even on different computers. This partly occurs as raw data is frequently unavailable with only preconfigured management traits being available to the user (Gengler, 2019). Many herd health systems fail to integrate data from different sources (Barkema *et al.*, 2015) due to a lack of inter-operable data standards (Bahlo *et al.*, 2019). It is not surprising therefore that farmers report concerns with data overload (Sørensen *et al.*, 2010). Although some effort has been made to develop systems that do integrate data these have mainly been in a research environment e.g. Nikander *et al.*, (2015). Another reason for this is the complex issues surrounding the ownership and use of data and thus the integration of data-streams (Buller *et al.*, 2020). There has been some moves by the industry to address these concerns (Wolfert *et al.*, 2017). It has been suggested that data should be available off-farm rather than simply being part of a closed loop on-farm (Gengler, 2019). However, companies may seek to control data (Wolf and Wood, 1997) and this is understandably of concern to producers. It is therefore not surprising that concerns have also been raised around how the use of these technologies re-distributes power between stakeholders (van der Burg *et al.*, 2019). Concerns have also

been raised about the possibilities of technology companies having monopolies over parts of the sector (Neethirajan, 2023). A further barrier to the use of disease detection tools on farm is the limited connectivity in rural areas, many tools are reliant on the internet to function (Selvaggi *et al.*, 2024).

Another concern is the lack of decision support tools available to accompany disease detection tools. Sun *et al.* (2021) highlighted that no systems appear to have decision support available. Decision support is defined as a system that aids the farmer to decide on an action e.g. whether to treat an animal and what for (Sun *et al.*, 2021). Decision support tools need to consider the framework proposed by Stachowicz and Umstatter (2021) as many currently on the market are detecting poor welfare not a specific disease. Although not on PLF decision support tools the finding of Rose *et al.* (2016) on factors surrounding decision support tool use should be considered. They found that performance, ease of use, peer recommendation, trust, cost and habit were all important parts of reasons behind the use of decision support tools by both farmers and their advisors. When developing decision support tools, it is vital to keep the end user in mind and there needs to be some flexibility as how they are used may change on a farm-by-farm basis (Rose *et al.*, 2018). Similarly, Eastwood *et al.* (2019) found that changes needed to be made in systems after installing them on farm, this included an automatic drafting gate to allow data produced by a body condition scoring system to be utilised by sorting cows for re-grouping.

There have been very few studies showing how the use of alerts helps calf outcomes and without this body of literature evidence-based decision support tools cannot be built. A recent study examined the response of calves treated with a non-steroidal anti-inflammatory (NSAID) following an alert for NCD. The authors found that there was no difference in growth rates between those that did or did not receive a NSAID following an alert. However, those that received a NSAID were less likely to be treated with electrolytes by blinded farm staff. Calves that had received an NSAID were also more active, lied down for less time and had more lying bouts than those that had not received an NSAID (Welk *et al.*, 2025). This may reflect a reduction in calves feeling malaise, which is considered negative for calf welfare (Millman, 2007). However, another recent study showed no difference between calves that received meloxicam or a placebo when they presented to the veterinary hospital for intravenous therapy (Neill

et al., 2025). This may be due to the late stage of disease as Todd *et al.* (2010) and Philipp *et al.* (2003) both found that NSAIDs improved calf outcomes. To this authors knowledge there are no studies examining the treatment success following an alert for BRD. It is likely that considerable work is needed in this area. A study by Mahendran *et al.* (2017) found that 25% of BRD cases resolved following NSAID treatment, however antimicrobials were still required in the remaining 75% of cases. Early detection may still reduce antimicrobial use, a recent on farm trial reduced antimicrobial use by 55% by using a commercially available BRD detection system (Arwain DGC, 2025). Thus, disease detection tools certainly have a place but more research to determine the interventions required when an alert is produced is required.

There are many considerations for the development of new disease detection systems. PLF technologies need to be able to cope with the rigours of being used on farm. This may include impact and extreme temperatures and humidity (Stachowicz and Umstatter, 2021). We must also ensure that these technologies meet the demands of farmers and do not take control from the farmer to the supplier of these technologies (Rose and Chilvers, 2018). Eastwood *et al.* (2022) developed a design guide for robotics in pasture-grazed cattle in New Zealand and this guide should be considered when designing disease detection systems. Key considerations include staff safety, ease of use, suitability for the cattle in the system, impact on self-identity of staff, health and safety and data integration. Although alluded to Eastwood *et al.* (2022) did not explicitly include impact on animal welfare in their considerations. This should be central to the development of any disease detection system in the future.

In conclusion further research to examine how the implementation of early disease detection systems influences calf welfare, health, growth, future production, and antibiotic use is needed. In order for tools to be used by the industry then trust is required and accuracy information should be produced to foster this as well as decision support systems.

Table 6.1 Studies on behaviour-based disease detection models in calves

Study	Disease definition	Daily milk allowance (litres)	Model type	Se (%)	Sp (%)	PPV (%)	NPV (%)
Bushby <i>et al.</i> (2024)	WCHS \geq 5	10	Gradient boosting	46	93	-	-
	WCHS \geq 5 and/or pyrexia		machine learning	58	89	-	-
	WCHS \geq 5 and/or pyrexia and/or treated by farm staff		with 229 features	57	90	-	-
	WCHS \geq 5		Gradient boosting	54	90	-	-
	WCHS \geq 5 and/or pyrexia		machine learning	63	87	-	-
	WCHS \geq 5 and/or pyrexia and/or treated by farm staff		with 30 features	59	90	-	-
Cantor <i>et al.</i> (2024)	Two days of faecal score 2 or one day of faecal score 3	15	Relative change in milk intake	87	13	-	-
		10		71	Not calculable	-	-
		15	Rolling dividends in milk intake	71	63	-	-
		10		33	Not calculable	-	-
		15	Rolling dividend in milk intake or drinking speed	86	50	-	-
		10		45	Not calculable	-	-
Perttu <i>et al.</i> (2024)	Weekly WCHS and farm staff treatments	9	Random forest	70	Not given	-	-
			Gradient boosting	73	Not given	-	-
Ghaffari <i>et al.</i> (2022)	Used a modified version of WCHS	Ad libitum	Deep convolutional neural networks	83	71	39	95
				82	87	31	99
Bowen <i>et al.</i> (2021)	WCHS \geq 5 and temperature score = 3	Ad libitum	Lying time moving average	43	97	35	98
			Random forest	35	95	23	97
			Lying time moving average and random forest	54	94	29	98
Conboy <i>et al.</i> (2021)	WCHS \geq 5 Faeces score \geq 2	6 - 11	Best linear unbiased prediction with parallel testing	82	26	-	-
				78	23	-	-
Knauer <i>et al.</i> (2018)	Cough, increased respiratory rate or effort. Faeces score \geq 2	9.4 on average	Cumulative sum statistical control charts	75	27	65	37

Se = Sensitivity, Sp = Specificity, PPV = Positive predictive value, NPV = Negative predictive value, WCHS = Wisconsin calf health score

6.8 The lack of standardised definitions of disease and age groups hinders both research and benchmarking which may reduce opportunities to improve.

As discussed in Chapters 1, 3 and 5. There is currently a lack of standardisation of both disease and mortality definitions not only in the scientific literature but also on farm. This issue has previously been highlighted in the review looking at predicting morbidity and mortality by Morrison *et al.* (2021). Standardising definitions would allow mortality and disease incidence to be used by producers, veterinary surgeons, and advisors to monitor and benchmark performance. Benchmarking has been shown to aid in

motivating and informing change on farm (Wilson *et al.*, 2023b, Sumner *et al.*, 2018), particularly in those producers that are data motivated (Wilson *et al.*, 2023b).

Furthermore, studies should clearly state the disease definitions used and use validated clinical scoring systems where possible (Plaugher and Cantor, 2025).

Santman-Berends *et al.* (2019) collaborated with experts to develop definitions for mortality. A key issue arising was the variety of age groups that mortality data is currently collected for. Standardising the age groups that mortality is recorded and reported for allows direct comparisons (Santman-Berends *et al.*, 2019). A further challenge is that frequently mortality is only recorded in those animals that have received an ear tag and the timing of this may vary according to the legislation in the country in question (Santman-Berends *et al.*, 2019, Hyde *et al.*, 2020). As the pre-weaning population on dairy farms is frequently dynamic due to births and male/dairy-beef calves leaving the farm using incidence rate rather than incidence risk for calculating mortality is preferable (Umaña Sedó *et al.*, 2023). A standardised “birth certificate” for calves alongside a scheme to define causes of death to allow farmers and vets to better record and analyse calf mortality data has been proposed in the literature (Lombard *et al.*, 2019). A scheme has been operating in Norway for some time, however treatments are estimated to be under-recorded by 40% (Gulliksen *et al.*, 2009). A Canadian scheme looking at recording events around birth was well received (Vasseur *et al.*, 2010). Not only is this of concern in terms of safe medicine use but it also means that neither the farmer nor their advisors know the extent of any disease problem. A novel approach to calculate the burden of all morbidity and mortality has recently been suggested by Ramos *et al.* (2025) who used a disability-adjusted life years approach on forty dairy farms. This approach may be beneficial but requires further research.

Not only is disease frequently poorly recorded but disease is frequently poorly defined, even in the research literature. Wilson *et al.* (2023a) performed a scoping review of papers looking at NCD. They found that a faecal consistency score was the most used metric (alone: 281/933, combined with another metric 241/933), however these studies used between one and seven levels, most commonly four levels were used (208/522). The authors had to eliminate many papers (566/1121) from their analysis due to their not being a clear disease definition. For BRD a recent scoping review found that clinical

scoring was the most used metric (Alone: 148/358, with another metric: 33/358) with physical examination being the second most common metric (alone: 91/358, with another metric: 71/358) (Churchill *et al.*, 2025). This author suggests using a measure associated with production outcomes such as the maximum consolidation depth or qTUS score as described by Jourquin *et al.* (2025) recently. For both BRD and NCD, some of the literature uses farm treatments to determine disease events, however this may not be accurate as farm staff treatment decisions and veterinary observations of clinical signs have been shown to differ (Olson *et al.*, 2019). Furthermore, as discussed above there is a lack of disease recording on farm, with only 11% of milk-recording farmers recording calf diseases in Ontario (van Staaveren *et al.*, 2023).

Finally, the lack of disease recording will impede genetic evaluation of the calf's susceptibility to disease. While heritability is low, there is a moderate genetic correlation between NCD and BRD and the current evaluated health trait heifer liveability (Neupane *et al.*, 2025). This would allow farmers to select for calves that are less susceptible to disease in the future. In conclusion, disease must be clearly defined and consistently reported to aid researchers, veterinary surgeons and producers to monitor and control disease.

6.9 Limit fed calves are hungry: limiting their behavioural response to challenge.

In this study calves were offered a maximum of seven litres of milk replacer each day, this is far lower than the volume calves will consume voluntarily if fed *ad libitum* (Prokop *et al.*, 2025). The association between nutrition and behavioural responses to disease in calves is discussed in sections 1.6.1.3 and 3.5. Nutrition also effects behaviour as discussed in section 1.6.2. Some of the differences in behavioural response to disease depending on a calf's nutritional status may be due to immune function as discussed in section 3.5.

The association between nutrition and immunity may explain why BRD incidence was reduced in calves fed a greater amount of milk solids in the study by Medrano-Galarza *et al.* (2018). Calves fed a higher level of nutrition (both energy and protein) recovered from diarrhoea quicker after being infected with *Cryptosporidium* (Ollivett *et al.*, 2012). These difference in immunity and disease rates also appear to influence mortality. A

farm feeding nine or more litres of milk or milk replacer has been associated with a lower mortality rate (Umaña Sedó *et al.*, 2024). Increased fat content of the milk replacer has been shown to be associated with reduced mortality (Urie *et al.*, 2018b). These health benefits may contribute to the production benefits of feeding more milk to calves. A review has found that calves fed more milk have greater average daily gain which is not unsurprising (Welk *et al.*, 2023). Furthermore, as suggested by the meta-analysis by Soberon and Van Amburgh (2013) increased growth pre-weaning may result in epi-genetic programming that increases milk yield in the first lactation. This is supported by the findings of the meta-analysis by Gelsinger *et al.* (2016).

Calves being hungry is a welfare concern. Hunger is a normal sensation that drives an animal's motivation to feed (Mellor and Stafford, 2004). However, when there is insufficient food to meet the animals need, particularly for an extended period, then hunger becomes a welfare issue (Mellor and Stafford, 2004). Hunger in limit fed calves is demonstrated by an increase in the number of unrewarded visits to the milk feeder when compared to those on a higher level of milk replacer (De Paula Vieira *et al.*, 2008, Welk *et al.*, 2023). Both hunger (Costa *et al.*, 2019, EFSA Panel on Animal Health and Animal Welfare, 2023) and inadequate nutrition (Sumner and von Keyserlingk, 2018) have been identified as concerns in artificially reared calf welfare. Nutrition is one of the domains in the widely used Five Domains model of animal welfare (Mellor *et al.*, 2020). While this thesis uses the five domains model to frame welfare concerns it should be remembered however that the five domains model is a way of thinking about animal welfare not a way of assessing it (Hampton *et al.*, 2023).

In a recent survey vets, farmers and agricultural representatives all ranked low levels of milk replacer highly as a welfare concern in UK calves (Smith *et al.*, 2025). Other research has highlighted that many farms do not reach the legal requirement to feed at least twice a day in calves less than four weeks of age (Palczynski *et al.*, 2020, Mahendran *et al.*, 2022). Calves fed a high milk allowance also show more play behaviours (Welk *et al.*, 2023). Play is thought to reflect higher welfare (Duve *et al.*, 2012) and the lack of opportunities to express play behaviours is a key welfare concern in all artificial-rearing systems (EFSA Panel on Animal Health and Animal Welfare, 2023). Thus, feeding a higher milk allowance is likely to improve calf welfare.

The volume of milk offered in the studies included in this thesis is lower than the recent recommendation of feeding 20% of a calf's bodyweight for the first four weeks of life (EFSA Panel on Animal Health and Animal Welfare, 2023) (equivalent of 8.5 L/day from birth for calves in this thesis). However, a UK survey with 216 respondents indicated that 52.8% of respondents fed less than seven litres of milk or milk replacer a day (Mahendran *et al.*, 2022), therefore the studies in this thesis are representative of the average UK farm. Worryingly, 13% of respondents fed a maximum of four litres or less of milk or milk replacer a day. This is not only a challenge in the UK. A Norwegian study found that 59.6% of farms fed less than eight litres of milk or milk replacer a day (Johnsen *et al.*, 2021). In an older study in the USA 70.4% of farms specializing in heifer rearing fed less than 4.7 litres of milk or milk replacer a day (Machado and Ballou, 2022). However, in farms rearing their own calves then only 25% of farms are feeding 4.7 litres or less of milk or milk replacer each day (Urie *et al.*, 2018a). Interestingly, more recent work has shown that 86% of farms sampled in British Columbia fed more than 8 L/day (Russel *et al.*, 2025). This may indicate that the advice to feed more milk is beginning to influence on-farm practices.

The availability of labour to carry out calf feeding can be poor (Sinnott *et al.*, 2021). One possibility to avoid this is to feed more milk at each feed. A concern raised by calf care staff is that there is a risk of abdominal discomfort and milk entering the rumen if calves are fed a greater milk volume (Svensson *et al.*, 2023). Calves will voluntarily drink over six litres of warm whole milk from a bottle without experiencing pain or any milk entering the rumen (Ellingsen *et al.*, 2016). Thus, providing warm milk is provided from a teat it is possible to increase meal size (Ellingsen *et al.*, 2016). Eight litres of milk replacer daily is certainly practically achievable even in calves fed manually twice a day (Svensson *et al.*, 2023, Sinnott *et al.*, 2021).

Traditionally, the fear of nutritional NCD has prevented the feeding of elevated levels of milk replacer. The evidence increasingly suggests that this is not the case (Welk *et al.*, 2023). Other studies have fed *ad libitum* milk replacer with no change in diarrhoea incidence (Jasper and Weary, 2002). In one study calves fed a higher level of milk replacer has less diarrhoea (Khan *et al.*, 2007b). In another study calves on a higher plane of nutrition had a faster resolution of diarrhoea (Ollivett *et al.*, 2012). The evidence also suggests that the composition of the milk replacer plays a role (Wilms *et*

al., 2022). Supplementation of the fatty acids' linoleic acid and α -linolenic acid improved immune function and growth (Garcia *et al.*, 2015). The essential amino acid L-glutamine reduces gut-permeability and markers of stress in calves (Ceja *et al.*, 2023). Calves fed milk replacer with a lower lactose and higher fat content had increased gastrointestinal permeability markers but decreased faecal scores (Amado *et al.*, 2019). In another study, calves fed a higher fat milk replacer had less health events (Berends *et al.*, 2020).

There is a common school of thought that increased milk allowance will decrease solid feed intake (Svensson *et al.*, 2025). Some of the research supports this e.g. Olagunju *et al.* (2024), and Kmicikewycz *et al.* (2013). However, Hosseini *et al.* (2019) found that calves fed eight litres of milk did not consume less solid feed than those fed four litres of milk in the first ten weeks of life (Hosseini *et al.*, 2019), a finding supported by Orellana Rivas *et al.* (2020). Gradual weaning of calves on higher levels of milk increases solid feed intake, calf growth, and rumen development (Khan *et al.*, 2007a). Provision of forage during the milk feeding stage will also aid in rumen development (Khan *et al.*, 2011). Weaning at eight weeks rather than six weeks reduces non-nutritive oral behaviours and increases lying and rumination time during the step-down phase (Eckert *et al.*, 2015). Beginning weaning at eighty days of age rather than forty days of age led to better growth, less unrewarded visits to the milk feeder and calves consuming more starter during the step-down phase (de Passillé and Rushen, 2016). This approach was supported by Fischer *et al.* (2019) in their review. While weaning according to starter intake is possible, no differences in unrewarded visits or ADG between late weaned calves and those weaned according to starter intake was seen by de Passillé and Rushen (2016). Thus, simply weaning later may be a simpler approach.

In conclusion, the nutritional status of the calves included in this study is likely to have influenced their behavioural response to disease but was reflective of UK practice. Further work needs to be done to ensure the information regarding feed size, nutritional NCD, solid feed intake and weaning is disseminated to producers. This would allow this significant welfare concern in pre-weaned artificially reared calves to be addressed.

6.10 Calves require social housing for high welfare.

Another key welfare challenge in calves is their social environment (Costa *et al.*, 2019). The provision of suitable social opportunities is part of the third domain of the Five Domains Model (Mellor *et al.*, 2020). The effect of individual versus pair or group housing on behaviour is discussed in section 1.6.2. Calves housed with others from an early age appear to cope better with a variety of stressors. Individually housed calves and late paired calves vocalize more after weaning than those paired from a week of age (Bolt *et al.*, 2017). This is supported by the findings of De Paula Vieira *et al.* (2010) and Overvest *et al.* (2018). Calves that were socially housed from birth appear to cope better with social regrouping (Lindner *et al.*, 2021, Bøe and Færevik, 2003). Pair housed calves respond better to handling than their individually housed counterparts (Duve *et al.*, 2012). Pair-housed calves have also been showed to be more positive when given ambiguous clues during a spatial go/no-go task, this optimism may reflect a positive affective state (Bučková *et al.*, 2019).

Calves have been shown to have greater motivation for full social contact than for head only contact (Holm *et al.*, 2002, Ede *et al.*, 2022). Evidence suggests that physical contact is required to develop social bonds in young dairy calves (Lindner *et al.*, 2022). Thus, housing which provides full social contact is preferable.

Despite popular belief there is increasing evidence that housing calves together does not affect disease incidence (Mahendran *et al.*, 2023b, Jensen and Larsen, 2014). Furthermore, this author agrees with Costa *et al.* (2016) that any risks can be mitigated and do not warrant the welfare cost of individual housing. Such measures might include using small and stable groups (Montes and Boerman, 2024, Abdelfattah *et al.*, 2024) and ensuring a high level of hygiene.

Social housing may benefit growth in the long-term, with calves that were pair housed pre-weaning being heavier at twelve months of age than those that were individually housed (Lindner *et al.*, 2025). Group housed calves were intermediate, possibly as more group housed calves had a fever pre-weaning (Lindner *et al.*, 2025). Another study found that group-housed calves gain more weight, play more and eat more concentrate (Donadio *et al.*, 2025). Not all studies agree that pair housed calves have better weight gain in the long term, Riesgraf *et al.* (2024) found no difference between calves that were pair housed or individually housed in the pre-weaned period at eighteen months

of age. In a study of Jersey calves, pair housed calves grew better than individually housed calves but cross-sucking was observed in 13.5% of pairs (Pempek *et al.*, 2016). Interestingly, an interaction between milk feeding and social environment may occur. Jensen *et al.*, (2015) found that pair housed calves on an enhanced milk feeding regime ate more concentrate and gained more weight than individually housed calves. However, this was not the case in the calves on a standard milk feeding regime (Jensen *et al.*, 2015). This may be related to interactions around milk feeding in calves on an automatic milk feeder. Calves fed 10% of their bodyweight daily were displaced from the feeder more often than those fed ad libitum (De Paula Vieira *et al.*, 2008).

Individually housed calves may have less opportunities to express active behaviours as they spend more time running during an arena test (Bučková *et al.*, 2021, De Paula Vieira *et al.*, 2012). This is likely due to the size of the pens as calves housed in a smaller space also spend more time running in an arena test (Sutherland *et al.*, 2014). When calves were confined to a smaller space both galloping and bucking were increased in an open-field test (Jensen, 1999) However, in another study neither individually nor group housed calves with a greater space allowance showed differing behaviours to calves with a smaller space-allowance in an open-field test (Jensen *et al.*, 1997). An increased space allowance has been associated with increased growth in individually housed calves (Calvo-Lorenzo *et al.*, 2016). Pair housing calves will allow an increase in a calf's living space of 35% while maintaining a gap between pens to limit pathogen transfer, however care needs to be taken to avoid cross-sucking and competition over resources (Mandel *et al.*, 2016). Cross-sucking represents a mis-guided oral behaviour, which are reduced in socially housed calves even when accounting for cross-sucking (Doyle and Miller-Cushon, 2023). Unsurprisingly, feeding more milk reduces the level of cross-sucking (Jung and Lidfors *et al.*, 2001, Nielsen *et al.*, 2008). Other measures to reduce cross-sucking include using a slow-flow teat bucket for feeding milk instead of an open bucket (Salter *et al.*, 2021), providing hay (Horvath *et al.*, 2020, Downey and Tucker, 2023) or brushes (Horvath *et al.*, 2020). Furthermore, some evidence suggests that cross-sucking may not be associated with udder health as traditionally thought (Vaughan *et al.*, 2016). In farms using hutches placing two hutches side by side and placing a pen to the front may be a simple way of providing pair housing (Whalin *et al.*, 2018).

It is important to consider that social housing can also be a source of stress. Social stressors will influence the immune system and thus disease, particularly in early life (Proudfoot *et al.*, 2012). Chronic or repeated social stressors will cause a dysfunction of the immune system (Proudfoot *et al.*, 2012). There is increased competition in calves housed in groups of six when compared to pair housed calves (Jensen and Budde, 2006). Minimizing introductions and the age-range within a group will reduce social stress and improve health and welfare. Thus, group size should be appropriate for the farms calving pattern. It is likely that small groups may provide the best opportunities to display natural behaviours (Barry *et al.*, 2020). However, this may not be suitable for automatic milking systems as farms using automatic milk feeders often have to place more calves in each pen to make the feeder cost-effective (Jensen, 2004). A survey in Canada suggested that both the size and age-range of groups were larger in calves using automatic milk feeders (Medrano-Galarza *et al.*, 2017). However, if group sizes become too large then access to the feeder becomes restricted (Brscic *et al.*, 2009). Feeding rate and the number of times a feeding bout is disturbed are increased when there is twenty-four rather than twelve calves per feeder (Jensen, 2004). Housing calves in larger groups will also increase the risk of respiratory disease (Svensson *et al.*, 2003, Svensson and Liberg, 2006). Calves in small groups of three to eight calves had lower mortality than those kept individually or in groups of six to thirty calves in one Swedish study (Svensson *et al.*, 2006). The risk of disease is increased if the group is dynamic (Barkema *et al.*, 2015).

As a heifer will encounter changes in social group, housing and management throughout her life providing her with the tools to cope with these changes is likely to be of considerable benefit to her welfare (Miller-Cushon, 2024). Furthermore, positive affective states have been associated with longevity (Walker *et al.*, 2012), thus well managed group housing may also increase a cows' productive lifetime. The recent European recommendation of pairs or small groups (< 8 calves) from the first week of life balances the benefits of social housing with its risks (EFSA Panel on Animal Health and Animal Welfare, 2023) and is consistent with the findings of the review by Miller-Cushon and Jensen (2025). Group size should be determined by the farms calving pattern so that groups can remain stable and have minimal age-range. Where systems

are being changed then extended support is needed to help producers overcome any challenges that arise from the change (Kuo *et al.*, 2025).

In this thesis calves were housed individually until approximately a week of age, they were then moved into a group pen. This is a common system in the UK with 20.8% of farmers moving calves from individual to group pens prior to weaning (Mahendran *et al.*, 2022). In this study the groups were larger than the average (seven calves) reported in the survey by Mahendran *et al.* (2022). The group size may have been too large thus increasing disease risk. In some cases, it took up to four weeks to fill a pen of approximately twelve calves. Partly due to milk buyer contracts less calves are housed individually in the UK than previously with 31.1% of farmers moving away from individual housing in the previous two years (Mahendran *et al.*, 2022). It should be considered whether more smaller group pens would be more suitable for this farm's calving pattern. However, due to the expense of automatic milk feeding systems this may not be possible.

In conclusion, individual housing is a significant welfare concern in calves. Greater work to promote small group housing should be carried out however this may make feeding larger volumes of milk more difficult as automatic milk feeders are costly.

6.11 How does the public perceive artificially reared calves?

While maintaining health, welfare and production of the animals in our care we must also consider the social license to farm. Recent years has seen an increase in both public and political interest in animal welfare (Ohl and van der Staay, 2012). While the industry has frequently used production and health as markers of high welfare the public frequently concentrate on affective states (Barkema *et al.*, 2015) and perceived naturalness (Barkema *et al.*, 2015. Cardoso *et al.*, 2016). It is therefore not surprising that group housing was considered preferable to individual housing in an American survey (Perttu *et al.* 2020). Social contact, and needs associated with biological functioning (e.g. food and water) are also considered important by the public (Perttu *et al.* 2021). However, a natural environment may risk an animal's welfare, due to exposure to the elements (Beaver *et al.*, 2019b). Indeed, calves' preference for outdoor access is dependent on the weather (Whalin *et al.*, 2022). It is worth considering that some of the topics discussed above such as social housing, increased milk allowance

and gradual weaning all represent a more “natural” approach to managing calves (Whalin *et al.*, 2021).

Cow-calf separation is rejected by many members of the public and this may prove a challenge to the dairy industry going forward (Hötzel *et al.*, 2017, Sirovica *et al.*, 2022, Busch *et al.*, 2017). Calves reared with their dam have a wider range of both exploratory and social behaviours (Beaver *et al.*, 2019b) and express less non-nutritive oral behaviours. Prolonged cow-calf contact appears to benefit the animal’s welfare, particularly that of the calf due to cognitive development benefits (Meagher *et al.*, 2019) without affecting their health (Beaver *et al.*, 2019a). There is considerable research into cow-calf contact systems in recent decades as reviewed by Whalin *et al.* (2025). It may well be that consumer pressure leads to an increase in these systems going forward. Further research is needed to explore how cow-calf systems can satisfy the needs of the cow, the calf and the farmer and the demands of the public (Nawroth and Rørvang, 2022).

It should be remembered that the public will have a range of concerns and we must address them all to ensure continued social license (von Keyserlingk *et al.*, 2009). Concerns may also vary by country (Evans and Miele, 2019). There is significant disconnect between the views of farmers and their advisors and the public (Cardoso *et al.*, 2019). However, farmers are aware that calf care poses a reputational risk to the industry and this is a motivator in improving care (Wilson *et al.*, 2024). Clear labelling is required so that consumers can make informed decisions regarding the products they are purchasing (Thibault *et al.*, 2022, Alonso *et al.*, 2020). There is some evidence that consumers are willing to pay more for higher welfare products in the USA (Spain *et al.*, 2018), Germany (Oehlmann *et al.*, 2026) and the UK (Gorton *et al.*, 2023). However, unsurprisingly socio-economic factors play a role (Gorton *et al.*, 2023).

Initiatives such as farm walks may aid in building consumer trust (Ferris *et al.*, 2020). However, some farmers have expressed concern over giving the public too much power over how farms are run (Ritter *et al.*, 2020) and there has been a suggestion that policymakers are out of touch with the realities of farming (Wilson *et al.*, 2024). This is reflected in the attitudes of some farmers towards policy changes requiring measures such as social housing (Koralesky *et al.*, 2025). In contrast the use of an advisory tool to score different areas of management led to voluntary improvements in calf welfare

(Vasseur *et al.*, 2010). However, this may not be representative as farmers had volunteered for the study, suggesting that they may have already desired to improve. Further work is needed to identify the barriers to change within the calf industry (Roche *et al.*, 2023). This will allow effective knowledge exchange and allow the industry to move forward and remain acceptable to the public.

In conclusion, calf management particularly cow-calf separation, social housing and feeding are areas where the industry may find increasing pressures on its social license. This must be considered in future research and industry initiatives,

6.12 General discussion conclusions

In conclusion, the use of technology, novel diagnostics and data analytics may improve the detection of infectious diseases in pre-weaned dairy bred calves. Sensitivity and specificity data should be published when marketing disease detection systems to allow advisors to give suitable advice to their clients. Research on decision support tools is also required. However, this should only be considered as part of wider measures to improve to calf welfare, including a greater milk allowance and providing social contact.

6.13 Final conclusions

Disease is a significant welfare problem in pre-weaned artificially reared calves. This thesis found that age, weight, sex and sire breed type are associated with healthy calf behaviour. Calves with BRD spend more time at the milk feeder after peak BRD and drink more milk per visit prior to peak BRD. Calves on a lower plane of nutrition appear to show fewer changes in behaviour with BRD than those on a higher plane of nutrition. Calf saliva pH is indicative of NCD but the mechanism is currently unclear as there is no relationship between saliva pH and haematocrit or strong ion difference. Calves with NCD are less active, have fewer and longer lying bouts, had longer visits to milk and were less likely to drink their milk allowance than their healthy counterparts. There may be an association between both age and season and response to disease but inferences are limited due to an unbalanced dataset.

In conclusion, further research is needed on both behaviour and saliva-based disease detection tools in calves. Research looking to reduce the rate of data loss, provide external validation and accuracy information, and understand how plane of nutrition

affects disease detection tools is required. This should be accompanied by knowledge exchange and behaviour change to improve nutrition and housing for calves so that we can meet the demands of all stakeholders, not least the needs of the calves themselves.

6.14 Bibliography

- ADIB, M. R., BARRETT, C., O'SULLIVAN, S., FLYNN, A., MCFADDEN, M., KENNEDY, E. & O'RIORDAN, A. 2025. In situ pH-Controlled electrochemical sensors for glucose and pH detection in calf saliva. *Biosensors and Bioelectronics*, 275, 117234.
- ABDELFATTAH, E. M., ALY, S. S., LEHENBAUER, T. W. & KARLE, B. M. 2024. Effects of simplified group housing on behavior, welfare, growth performance, and health of preweaned dairy calves on a California dairy. *Journal of Dairy Science*, 107, 3927-3940.
- ALONSO, M. E., GONZÁLEZ-MONTAÑA, J. R. & LOMILLOS, J. M. 2020. Consumers' concerns and perceptions of farm animal welfare. *Animals*, 10.
- ANIMAL AND PLANT HEALTH AGENCY & SCOTTISH AGRICULTURE COLLEGE VETERINARY SERVICES 2017. Alimentary and respiratory disease trends for calves, 2011 to 2016. *Veterinary Record*, 180, 144-145.
- ARWAIN DGC. 2025. Proof of Concept Farms 2025 Booklet. Proof-of-Concept-Farms-2025-Booklet.pdf
- BAHLO, C., DAHLHAUS, P., THOMPSON, H. & TROTTER, M. 2019. The role of interoperable data standards in precision livestock farming in extensive livestock systems: A review. *Computers and Electronics in Agriculture*, 156, 459-466.
- BALLOU, M. A. 2012. Immune responses of Holstein and Jersey calves during the preweaning and immediate postweaned periods when fed varying planes of milk replacer. *Journal of Dairy Science*, 95, 7319-30.
- BARHEMA, H. W., VON KEYSERLINGK, M. A. G., KASTELIC, J. P., LAM, T. J. G. M., LUBY, C., ROY, J. P., LEBLANC, S. J., KEEFE, G. P. & KELTON, D. F. 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. *Journal of Dairy Science*, 98, 7426-7445.
- BARRY, J., BOKKERS, E. A. M., DE BOER, I. J. M. & KENNEDY, E. 2020. Pre-weaning management of calves on commercial dairy farms and its influence on calf welfare and mortality. *Animal*, 14, 2580-2587.
- BEAVER, A., MEAGHER, R. K., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2019a. Invited review: A systematic review of the effects of early separation on dairy cow and calf health. *Journal of Dairy Science*, 102, 5784-5810.
- BEAVER, A., RITTER, C. & VON KEYSERLINGK, M. A. G. 2019b. The Dairy Cattle Housing Dilemma: Natural Behavior Versus Animal Care. *Veterinary Clinics of North America: Food Animal Practice*, 35, 11-27.

- BØE, K. E. & FÆREVIK, G. 2003. Grouping and social preferences in calves, heifers and cows. *Applied Animal Behaviour Science*, 80, 175-190.
- BOLT, S. L., BOYLAND, N. K., MLYNSKI, D. T., JAMES, R. & CROFT, D. P. 2017. Pair Housing of Dairy Calves and Age at Pairing: Effects on Weaning Stress, Health, Production and Social Networks. *PLOS ONE*, 12, e0166926.
- BOWEN, J. M., HASKELL, M. J., MILLER, G. A., MASON, C. S., BELL, D. J. & DUTHIE, C. A. 2021. Early prediction of respiratory disease in preweaning dairy calves using feeding and activity behaviors. *Journal of Dairy Science*, 104, 12009-12018.
- BUČKOVÁ, K., ŠÁROVÁ, R., MORAVCSÍKOVÁ, Á. & ŠPINKA, M. 2021. The effect of pair housing on dairy calf health, performance, and behavior. *Journal of Dairy Science*, 104, 10282-10290.
- BUČKOVÁ, K., ŠPINKA, M. & HINTZE, S. 2019. Pair housing makes calves more optimistic. *Scientific Reports*, 9, 20246.
- BUCZINSKI, S. & PARDON, B. 2020. Bovine Respiratory Disease Diagnosis: What Progress Has Been Made in Clinical Diagnosis? *Veterinary Clinics of North America: Food Animal Practice*, 36, 399-423.
- BUSCH, G., WEARY, D. M., SPILLER, A. & VON KEYSERLINGK, M. A. G. 2017. American and German attitudes towards cow-calf separation on dairy farms. *PLOS ONE*, 12, e0174013.
- BUSHBY, E. V., THOMAS, M., VÁZQUEZ-DIOSDADO, J. A., OCCHIUTO, F. & KALER, J. 2024. Early detection of bovine respiratory disease in pre-weaned dairy calves using sensor based feeding, movement, and social behavioural data. *Scientific Reports*, 14, 9737.
- CALVO-LORENZO, M. S., HULBERT, L. E., FOWLER, A. L., LOUIE, A., GERSHWIN, L. J., PINKERTON, K. E., BALLOU, M. A., KLASING, K. C. & MITLOEHNER, F. M. 2016. Wooden hutch space allowance influences male Holstein calf health, performance, daily lying time, and respiratory immunity. *Journal of Dairy Science*, 99, 4678-4692.
- CANTOR, M. C., WELK, A. A., CREUTZINGER, K. C., WOODRUM SETSER, M. M., COSTA, J. H. C. & RENAUD, D. L. 2024. The development and validation of a milk feeding behavior alert from automated feeder data to classify calves at risk for a diarrhea bout: A diagnostic accuracy study. *Journal of Dairy Science*, 107, 3140-3156.
- CARDOSO, C. S., HÖTZEL, M. J., WEARY, D. M., ROBBINS, J. A. & VON KEYSERLINGK, M. A. G. 2016. Imagining the ideal dairy farm. *Journal of Dairy Science*, 99, 1663-1671.
- CARDOSO, C. S., VON KEYSERLINGK, M. G. & HÖTZEL, M. J. 2019. Views of dairy farmers, agricultural advisors, and lay citizens on the ideal dairy farm. *Journal of Dairy Science*, 102, 1811-1821.
- CEJA, G., BOERMAN, J. P., NEVES, R. C., JORGENSEN, M. W. & JOHNSON, J. S. 2023. L-Glutamine supplementation reduces gastrointestinal permeability and biomarkers of

physiological stress in preweaning Holstein heifer calves. *Journal of Dairy Science*, 106, 9663-9676.

CHERNITSKIY, A. E. & SAFONOV, V. A. Early detection of bovine respiratory disease in calves by induced cough. In: KOVALEV, I. V., PYZHIKOVA, N. I., VOROSHILOVA, A. A. & SHAPOROVA, Z. E., eds., 2021. IOP Publishing Ltd.

CHURCHILL, K. J., WINDER, C. B., GOETZ, H. M., WILSON, D., UYAMA, T., PARDON, B. & RENAUD, D. L. 2025. Evaluating case definitions of respiratory disease in dairy calves: A scoping review. *Journal of Dairy Science*, 108, 4030-4048.

CONBOY, M. H., WINDER, C. B., MEDRANO-GALARZA, C., LEBLANC, S. J., HALEY, D. B., COSTA, J. H. C., STEELE, M. A. & RENAUD, D. L. 2021. Associations between feeding behaviors collected from an automated milk feeder and disease in group-housed dairy calves in Ontario: A cross-sectional study. *Journal of Dairy Science*, 104, 10183-10193.

COSTA, J. H. C., CANTOR, M. C., ADDERLEY, N. A., NEAVE, H. W. & MIGLIOR, F. 2019. Key animal welfare issues in commercially raised dairy calves: social environment, nutrition, and painful procedures. *Canadian Journal of Animal Science*, 99, 649-660.

COSTA, J. H. C., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2016. Invited review: Effects of group housing of dairy calves on behavior, cognition, performance, and health. *Journal of Dairy Science*, 99, 2453-2467.

CRAMER, C. & OLLIVETT, T. L. 2020. Behavior assessment and applications for BRD diagnosis: preweaned dairy calves. *Animal Health Research Reviews*, 21, 188-191.

CRAMER, C., PROUDFOOT, K. & OLLIVETT, T. 2020. Automated Feeding Behaviors Associated with Subclinical Respiratory Disease in Preweaned Dairy Calves. *Animals*, 10, 1-9.

DAWKINS, M. S. 2025. Smart farming and Artificial Intelligence (AI): How can we ensure that animal welfare is a priority? *Applied Animal Behaviour Science*, 283, 106519.

DE PASSILLÉ, A. M. & RUSHEN, J. 2016. Using automated feeders to wean calves fed large amounts of milk according to their ability to eat solid feed. *Journal of Dairy Science*, 99, 3578-3583.

DE PAULA VIEIRA, A., GUESDON, V., DE PASSILLÉ, A. M., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2008. Behavioural indicators of hunger in dairy calves. *Applied Animal Behaviour Science*, 109, 180-189.

DE PAULA VIEIRA, A., DE PASSILLE, A. M. & WEARY, D. M. 2012. Effects of the early social environment on behavioral responses of dairy calves to novel events. *Journal of Dairy Science*, 95, 5149-5155.

DE PAULA VIEIRA, A., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2010. Effects of pair versus single housing on performance and behavior of dairy calves before and after weaning from milk. *Journal of Dairy Science*, 93, 3079-3085.

DOIDGE, C., ÅNESTAD, L. M., BURRELL, A., FRÖSSLING, J., PALCZYNSKI, L., PARDON, B., VELDHUIS, A., BOKMA, J., CARMO, L. P., HOPP, P., GUELBENZU-GONZALO, M., MEUNIER, N. V., ORDELL, A., SANTMAN-BERENDS, I., VAN SCHAİK, G. & KALER, J. 2024. A Living Lab approach to understanding dairy farmers' technology and data needs to improve herd health: Focus groups from 6 European countries. *Journal of Dairy Science*, 107, 5754-5778.

DONADIO, J. P., DE-SOUSA, K. T., TORRES, R. D. N. S., ALVES, T. C., HÖTZEL, M. J. & DENIZ, M. 2025. A meta-analysis approach to evaluate the effects of early group housing on calf performance, health, and behavior during the preweaning period. *Journal of Dairy Science*, 108, 954-967.

DOWNEY, B. C. & TUCKER, C. B. 2023. Providing long hay in a novel pipe feeder or a bucket reduces abnormal oral behaviors in milk-fed dairy calves. *Journal of Dairy Science*, 106, 1968-1985.

DOYLE, S. B. & MILLER-CUSHON, E. K. 2023. Influences of human contact following milk-feeding on nonnutritive oral behavior and rest of individual and pair-housed dairy calves during weaning. *JDS Communications*, 4, 46-50.

DUTHIE, C. A., BOWEN, J. M., BELL, D. J., MILLER, G. A., MASON, C. & HASKELL, M. J. 2021. Feeding behaviour and activity as early indicators of disease in pre-weaned dairy calves. *Animal*, 15, 100150.

DUVE, L. R., WEARY, D. M., HALEKOH, U. & JENSEN, M. B. 2012. The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. *Journal of Dairy Science*, 95, 6571-81.

EASTWOOD, C., AYRE, M., NETTLE, R. & DELA RUE, B. 2019. Making sense in the cloud: Farm advisory services in a smart farming future. *NJAS: Wageningen Journal of Life Sciences*, 90-91, 1-10.

EASTWOOD, C., KLERKX, L. & NETTLE, R. 2017. Dynamics and distribution of public and private research and extension roles for technological innovation and diffusion: Case studies of the implementation and adaptation of precision farming technologies. *Journal of Rural Studies*, 49, 1-12.

EASTWOOD, C. R., DELA RUE, B., EDWARDS, J. P. & JAGO, J. 2022. Responsible robotics design—A systems approach to developing design guides for robotics in pasture-grazed dairy farming. *Frontiers in Robotics and AI*, Volume 9 - 2022.

EASTWOOD, C. R. & RENWICK, A. 2020. Innovation Uncertainty Impacts the Adoption of Smarter Farming Approaches. *Frontiers in Sustainable Food Systems*, Volume 4 - 2020.

ECKELKAMP, E. A. 2019. Invited Review: Current state of wearable precision dairy technologies in disease detection. *Applied Animal Science*, 35, 209-220.

ECKELKAMP, E. A. & BEWLEY, J. M. 2020. On-farm use of disease alerts generated by precision dairy technology. *Journal of Dairy Science*, 103, 1566-1582.

- ECKERT, E., BROWN, H. E., LESLIE, K. E., DEVRIES, T. J. & STEELE, M. A. 2015. Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage. *Journal of Dairy Science*, 98, 6315-26.
- EDE, T., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2022. Calves are socially motivated. *JDS Communications*, 3, 44-48.
- EFSA PANEL ON ANIMAL HEALTH AND ANIMAL WELFARE, NIELSEN, S. S., ALVAREZ, J., BICOUT, D. J., CALISTRI, P., CANALI, E., DREWE, J. A., GARIN-BASTUJI, B., GONZALES ROJAS, J. L., GORTAZAR SCHMIDT, C., HERSKIN, M., MICHEL, V., MIRANDA CHUECA, M. A., PADALINO, B., PASQUALI, P., ROBERTS, H. C., SPOOLDER, H., STAHL, K., VELARDE, A., VILTROP, A., JENSEN, M. B., WAIBLINGER, S., CANDIANI, D., LIMA, E., MOSBACH-SCHULZ, O., VAN DER STEDE, Y., VITALI, M. & WINCKLER, C. 2023. Welfare of calves. *EFSA Journal*, 21, e07896.
- ELLINGSEN, K., MEJDELL, C. M., OTTESEN, N., LARSEN, S. & GRØNDAHL, A. M. 2016. The effect of large milk meals on digestive physiology and behaviour in dairy calves. *Physiology & Behavior*, 154, 169-174.
- EVANS, A. B. & MIELE, M. 2019. Enacting public understandings: The case of farm animal welfare. *Geoforum*, 99, 1-10.
- FERRIS, T. A., SMITH, J. M., DUNCKEL, M. A., CULLENS, F. & KUSCHEL, A. 2020. Designing educational farm tours to improve consumer trust in modern agriculture. *Journal of Extension*, 58, 1-7.
- FISCHER, A. J., VILLOT, C., VAN NIEKERK, J. K., YOHE, T. T., RENAUD, D. L. & STEELE, M. A. 2019. Invited Review: Nutritional regulation of gut function in dairy calves: From colostrum to weaning. *Applied Animal Science*, 35, 498-510.
- GARCIA, M., SHIN, J. H., SCHLAEFLI, A., GRECO, L. F., MAUNSELL, F. P., SANTOS, J. E. P., STAPLES, C. R. & THATCHER, W. W. 2015. Increasing intake of essential fatty acids from milk replacer benefits performance, immune responses, and health of preweaned Holstein calves. *Journal of Dairy Science*, 98, 458-477.
- GELSINGER, S. L., HEINRICHS, A. J. & JONES, C. M. 2016. A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance¹. *Journal of Dairy Science*, 99, 6206-6214.
- GENGLER, N. 2019. Symposium review: Challenges and opportunities for evaluating and using the genetic potential of dairy cattle in the new era of sensor data from automation. *Journal of Dairy Science*, 102, 5756-5763.
- GHAFFARI, M. H., MONNERET, A., HAMMON, H. M., POST, C., MÜLLER, U., FRIETEN, D., GERBERT, C., DUSEL, G. & KOCH, C. 2022. Deep convolutional neural networks for the detection of diarrhea and respiratory disease in preweaning dairy calves using data from automated milk feeders. *Journal of Dairy Science*, 105, 9882-9895.

- GOMEZ, D. E., LOFSTEDT, J., STAMPFLI, H. R., WICHTEL, M., MUIRHEAD, T. & MCCLURE, J. T. 2013. Contribution of unmeasured anions to acid-base disorders and its association with altered demeanor in 264 calves with neonatal diarrhea. *Journal of Veterinary Internal Medicine*, 27, 1604-12.
- GORTON, M., YEH, C.-H., CHATZOPOULOU, E., WHITE, J., TOCCO, B., HUBBARD, C. & HALLAM, F. 2023. Consumers' willingness to pay for an animal welfare food label. *Ecological Economics*, 209, 107852.
- GULLIKSEN, S. M., LIE, K. I. & ØSTERÅS, O. 2009. Calf health monitoring in Norwegian dairy herds. *Journal of Dairy Science*, 92, 1660-1669.
- HAMPTON, J. O., HEMSWORTH, L. M., HEMSWORTH, P. H., HYNDMAN, T. H. & SANDØE, P. 2023. Rethinking the utility of the Five Domains model. *Animal Welfare*, 32, e62.
- HELD, S. D. E. & ŠPINKA, M. 2011. Animal play and animal welfare. *Animal Behaviour*, 81, 891-899.
- HIXSON, C. L., KRAWCZEL, P. D., CALDWELL, J. M. & MILLER-CUSHON, E. K. 2018. Behavioral changes in group-housed dairy calves infected with *Mannheimia haemolytica*. *Journal of Dairy Science*, 101, 10351-10360.
- HOLM, L., JENSEN, M. B. & JEPPESEN, L. L. 2002. Calves' motivation for access to two different types of social contact measured by operant conditioning. *Applied Animal Behaviour Science*, 79, 175-194.
- HORVATH, K. C. & MILLER-CUSHON, E. K. 2017. The effect of milk-feeding method and hay provision on the development of feeding behavior and non-nutritive oral behavior of dairy calves. *Journal of Dairy Science*, 100, 3949-3957.
- HORVATH, K. C. & MILLER-CUSHON, E. K. 2017. The effect of milk-feeding method and hay provision on the development of feeding behavior and non-nutritive oral behavior of dairy calves. *Journal of Dairy Science*, 100, 3949-3957.
- HOSSEINI, S. H., MIRZAEI-ALAMOUTI, H., VAZIRIGOHAR, M., MAHJOUBI, E. & REZAMAND, P. 2019. Effects of whole milk feeding rate and straw level of starter feed on performance, rumen fermentation, blood metabolites, structural growth, and feeding behavior of Holstein calves. *Animal Feed Science and Technology*, 255, 114238.
- HÖTZEL, M. J., CARDOSO, C. S., ROSLINDO, A. & VON KEYSERLINGK, M. A. G. 2017. Citizens' views on the practices of zero-grazing and cow-calf separation in the dairy industry: Does providing information increase acceptability? *Journal of Dairy Science*, 100, 4150-4160.
- HUHTANEN, P., RAMIN, M. & HRISTOV, A. N. 2019. Enteric methane emission can be reliably measured by the GreenFeed monitoring unit. *Livestock Science*, 222, 31-40. during weaning. *JDS Communications*, 4, 46-50.

- JENSEN, M. B. 1999. Effects of confinement on rebounds of locomotor behaviour of calves and heifers, and the spatial preferences of calves. *Applied Animal Behaviour Science*, 62, 43-56.
- JENSEN, M. B. 2004. Computer-Controlled Milk Feeding of Dairy Calves: The Effects of Number of Calves per Feeder and Number of Milk Portions on Use of Feeder and Social Behavior. *Journal of Dairy Science*, 87, 3428-3438.
- JENSEN, M. B. & BUDDE, M. 2006. The Effects of Milk Feeding Method and Group Size on Feeding Behavior and Cross-Sucking in Group-Housed Dairy Calves. *Journal of Dairy Science*, 89, 4778-4783.
- JENSEN, M. B., DUVE, L. R. & WEARY, D. M. 2015. Pair housing and enhanced milk allowance increase play behavior and improve performance in dairy calves. *Journal of Dairy Science*, 98, 2568-75.
- JENSEN, M. B. & LARSEN, L. E. 2014. Effects of level of social contact on dairy calf behavior and health. *Journal of Dairy Science*, 97, 5035-44.
- JENSEN, M. B., VESTERGAARD, K. S., KROHN, C. C. & MUNKSGAARD, L. 1997. Effect of single versus group housing and space allowance on responses of calves during open-field tests. *Applied Animal Behaviour Science*, 54, 109-121.
- JOHNSEN, J. F., HOLM, Y. I., MEJDELL, C. M., ELLINGSEN-DALSKAU, K., OSTERAS, O., A, D. S., SKJERVE, E. & A, N. D. 2021. A cross-sectional study of associations between herd-level calf mortality rates, compliance with legislation on calf welfare, and milk feeding management in Norwegian dairy herds. *Journal of Dairy Science*, 104, 839-848.
- JOHNSON, K. F., CHANCELLOR, N., BURN, C. C. & WATHES, D. C. 2017. Prospective cohort study to assess rates of contagious disease in pre-weaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. *Veterinary Record Open*, 4, e000226.
- JOHNSTON, D., KENNY, D. A., MCGEE, M., WATERS, S. M., KELLY, A. K. & EARLEY, B. 2016. Electronic feeding behavioural data as indicators of health status in dairy calves. *Irish Journal of Agricultural and Food Research*, 55, 159-168.
- JUNG, J. & LIDFORS, L. 2001. Effects of amount of milk, milk flow and access to a rubber teat on cross-sucking and non-nutritive sucking in dairy calves. *Applied Animal Behaviour Science*, 72, 201-213.
- KHAN, M. A., LEE, H. J., LEE, W. S., KIM, H. S., KI, K. S., HUR, T. Y., SUH, G. H., KANG, S. J. & CHOI, Y. J. 2007a. Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. *Journal of Dairy Science*, 90, 3376-87.
- KHAN, M. A., WEARY, D. M. & VON KEYSERLINGK, M. A. 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. *Journal of Dairy Science*, 94, 3547-53.

KHAN, M. A., WEARY, D. M. & VON KEYSERLINGK, M. A. 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. *Journal of Dairy Science*, 94, 3547-53.

KMICIKIEWYCZ, A. D., DA SILVA, D. N. L., LINN, J. G. & LITHERLAND, N. B. 2013. Effects of milk replacer program fed 2 or 4 times daily on nutrient intake and calf growth. *Journal of Dairy Science*, 96, 1125-1134.

KNAUER, W. A., GODDEN, S. M., DIETRICH, A., HAWKINS, D. M. & JAMES, R. E. 2018. Evaluation of applying statistical process control techniques to daily average feeding behaviors to detect disease in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, 101, 8135-8145.

KNAUER, W. A., GODDEN, S. M., DIETRICH, A. & JAMES, R. E. 2017. The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, 100, 5642-5652.

KUO, C., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2025. Dairy farmers' experiences with adopting social housing for milk-fed dairy calves. *Journal of Dairy Science*, 108, 13425-13438.

LAWRENCE, A. B., BROWN, S. M., BRADFORD, B. M., MABBOTT, N. A., BOMBAIL, V. & RUTHERFORD, K. M. D. 2025. Non-neuronal brain biology and its relevance to animal welfare. *Neuroscience & Biobehavioral Reviews*, 173, 106136.

LINDNER, E. E., BONNEY-KING, J., BURNER, C. M., KRAWCZEL, P. D., BROMFIELD, J. J., BITTAR, J. H. J., MARTINS, T. & MILLER-CUSHON, E. K. 2025. Long-term effects of preweaning social housing on growth and reproductive development of dairy heifers. *Journal of Dairy Science*, 108, 10023-10036.

LINDNER, E. E., GINGERICH, K. N., BURKE, K. C., DOYLE, S. B. & MILLER-CUSHON, E. K. 2022. Effects of Social Housing on Dairy Calf Social Bonding. *Animals (Basel)*, 12, 821.

LINDNER, E. E., GINGERICH, K. N. & MILLER-CUSHON, E. K. 2021. Effects of early social contact on dairy calf response to initial social grouping and regrouping. *Journal of Dairy Science*, 104, 10090-10099.

LITTIN, K., ACEVEDO, A., BROWNE, W., EDGAR, J., MENDEL, M., OWEN, D., SHERWIN, C., WÜRBEL, H. & NICOL, C. 2008. Towards humane end points: behavioural changes precede clinical signs of disease in a Huntington's disease model. *Proceedings of the Royal Society B: Biological Sciences*, 275, 1865-1874.

LOMBARD, J. E., GARRY, F. B., URIE, N. J., MCGUIRK, S. M., GODDEN, S. M., STERNER, K., EARLEYWINE, T. J., CATHERMAN, D. & MAAS, J. 2019. Proposed dairy calf birth certificate data and death loss categorization scheme. *Journal of Dairy Science*, 102, 4704-4712.

- LOWE, G. L., SUTHERLAND, M. A., WAAS, J. R., SCHAEFER, A. L., COX, N. R. & STEWART, M. 2019. Physiological and behavioral responses as indicators for early disease detection in dairy calves. *Journal of Dairy Science*, 102, 5389-5402.
- LOWIE, T., VANDEWALLE, J., HANLEY-COOK, G., PARDON, B. & BOKMA, J. 2024. Short communication: Circadian variations and day-to-day variability of clinical signs used for the early diagnosis of pneumonia within and between calves. *Research in Veterinary Science*, 166, 105082.
- MACHADO, V. S. & BALLOU, M. A. 2022. Overview of common practices in calf raising facilities. *Translational Animal Science*, 6, txab234.
- MAHENDRAN, S. A., BOOTH, R., BELL, N. J. & BURGE, M. 2017. Randomised positive control trial of NSAID and antimicrobial treatment for calf fever caused by pneumonia. *Veterinary Record*, 181, 45.
- MAHENDRAN, S. A., WATHES, D. C., BLACKIE, N. & BOOTH, R. E. 2023a. Calf Health, Feeding and Social Behaviours within Groups Fed on Automatic Milk Feeders. *Ruminants* [Online], 3.
- MAHENDRAN, S. A., WATHES, D. C., BOOTH, R. E., BAKER, N. & BLACKIE, N. 2023b. Effects of Individual and Pair Housing of Calves on Short-Term Health and Behaviour on a UK Commercial Dairy Farm. *Animals*, 13, 2140.
- MAHENDRAN, S. A., WATHES, D. C., BOOTH, R. E. & BLACKIE, N. 2022. A survey of calf management practices and farmer perceptions of calf housing in UK dairy herds. *Journal of Dairy Science*, 105, 409-423.
- MANDEL, R., NICOL, C. J., WHAY, H. R. & KLEMENT, E. 2017. Short communication: Detection and monitoring of metritis in dairy cows using an automated grooming device. *Journal of Dairy Science*, 100, 5724-5728.
- MCKAY, C., ELLIS, K., HASKELL, M. J. & GLADDEN, N. 2025. Impact of early social housing on the play behavior of neonatal and post-weaning dairy calves. *Frontiers in Veterinary Science*, Volume 12 - 2025.
- MEAGHER, R. K., BEAVER, A., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2019. Invited review: A systematic review of the effects of prolonged cow–calf contact on behavior, welfare, and productivity. *Journal of Dairy Science*, 102, 5765-5783.
- MEDRANO-GALARZA, C., LEBLANC, S. J., DEVRIES, T. J., JONES-BITTON, A., RUSHEN, J., MARIE DE PASSILLÉ, A. & HALEY, D. B. 2017. A survey of dairy calf management practices among farms using manual and automated milk feeding systems in Canada. *Journal of Dairy Science*, 100, 6872-6884.
- MEDRANO-GALARZA, C., LEBLANC, S. J., JONES-BITTON, A., DEVRIES, T. J., RUSHEN, J., MARIE DE PASSILLE, A., ENDRES, M. I. & HALEY, D. B. 2018. Associations between management practices and within-pen prevalence of calf diarrhea and respiratory

disease on dairy farms using automated milk feeders. *Journal of Dairy Science*, 101, 2293-2308.

MELCHNER, A., VAN DE BERG, S., SCUDA, N., FEUERSTEIN, A., HANCZARUK, M., SCHUMACHER, M., STRAUBINGER, R. K., MAROSEVIC, D. & RIEHM, J. M. 2021. Antimicrobial Resistance in Isolates from Cattle with Bovine Respiratory Disease in Bavaria, Germany. *Antibiotics*, 10.

MELLOR, D. J., BEAUSOLEIL, N. J., LITTLEWOOD, K. E., MCLEAN, A. N., MCGREEVY, P. D., JONES, B. & WILKINS, C. 2020. The 2020 Five Domains Model: Including Human–Animal Interactions in Assessments of Animal Welfare. *Animals* [Online], 10.

MELLOR, D. J. & STAFFORD, K. J. 2004. Animal welfare implications of neonatal mortality and morbidity in farm animals. *The Veterinary Journal*, 168, 118-133.

MIJARES, S., EDWARDS-CALLAWAY, L., ROMAN-MUNIZ, I. N., COETZEE, J. F., APPLGATE, T. J. & CRAMER, M. C. 2023. Veterinarians' perspectives of pain, treatment, and diagnostics for bovine respiratory disease in preweaned dairy calves. *Frontiers in Pain Research*, 4.

MILLER-CUSHON, E. K. 2024. Current research considering social behavior to improve the welfare of commercially raised dairy calves. *JDS Communications*, 5, 264-269.

MILLER-CUSHON, E. K. & DEVRIES, T. J. 2016. Effect of social housing on the development of feeding behavior and social feeding preferences of dairy calves. *Journal of Dairy Science*, 99, 1406-1417.

MILLER-CUSHON, E. K. & JENSEN, M. B. 2025. Invited review: Social housing of dairy calves—Management factors affecting calf behavior, performance, and health; A systematic review. *Journal of Dairy Science*, 108, 3019-3044.

MILLMAN, S. T. 2007. Sickness behaviour and its relevance to animal welfare assessment at the group level. *Animal Welfare*, 16, 123-125.

MONTES, M. E. & BOERMAN, J. P. 2024. Graduate Student Literature Review: Social and feeding behavior of group-housed dairy calves in automated milk feeding systems. *Journal of Dairy Science*, 107, 4833-4843.

MORRISON, J., RENAUD, D. L., CHURCHILL, K. J., COSTA, J. H. C., STEELE, M. A. & WINDER, C. B. 2021. Predicting morbidity and mortality using automated milk feeders: A scoping review. *Journal of Dairy Science*, 104, 7177-7194.

NAWROTH, C. & RØRVANG, M. V. 2022. Opportunities (and challenges) in dairy cattle cognition research: A key area needed to design future high welfare housing systems. *Applied Animal Behaviour Science*, 255, 105727.

NEETHIRAJAN, S. 2023. The Significance and Ethics of Digital Livestock Farming. *AgriEngineering* [Online], 5.

NEILL, H. M., REPERT, E. J., KLEINHENZ, M. D., WEEDER, M. M., SCHWARTZ, D., BEAR, M., KOMPALAGE, K., TUCKER, R., COETZEE, J. F. & VISCARDI, A. V. 2025. Evaluation of

oral meloxicam in beef calves with neonatal calf diarrhea. *Journal of the American Veterinary Medical Association*, 263, 1287-1295.

NEUPANE, M., PARKER GADDIS, K. L., TOGHIANI, S., MILES, A. M., GRAHAM, J. R., BURCHARD, J. F., DÜRR, J. W., COLE, J. B., O'CONNELL, J. R., VAN TASSELL, C. P. & VANRADEN, P. M. 2025. Improving dairy calf health through inclusion of diarrhea and respiratory health data into a US national genetic evaluation. *Journal of Dairy Science*, 108, 11164-11172.

NICOL, C. J. 2025. Behavioural indicators of infectious disease in managed animals. *Applied Animal Behaviour Science*, 285, 106573.

NIELSEN, P. P., JENSEN, M. B. & LIDFORS, L. 2008. Milk allowance and weaning method affect the use of a computer controlled milk feeder and the development of cross-sucking in dairy calves. *Applied Animal Behaviour Science*, 109, 223-237.

NIKANDER, J., LAAJALAHTI, M., KAJAVA, S., SAIRANEN, A., JÄRVINEN, M. & PASTELL, M. Development of a general cowshed information management system from proprietary subsystems. Precision Livestock Farming 2015 - Papers Presented at the 7th European Conference on Precision Livestock Farming, ECPLF 2015, 2015. 318-326.

OBEIDAT, B. S., COBB, C. J., SELLERS, M. D., PEPPER-YOWELL, A. R., EARLEYWINE, T. J. & BALLOU, M. A. 2013. Plane of nutrition during the preweaning period but not the grower phase influences the neutrophil activity of Holstein calves. *Journal of Dairy Science*, 96, 7155-7166.

OEHLMANN, M., DOLGOPOLOVA, I., NEUBIG, C. M. & ROOSEN, J. 2026. Willingness to pay for animal welfare across labels, products, consumers, and time. *Food Quality and Preference*, 135, 105708.

OHL, F. & VAN DER STAAY, F. J. 2012. Animal welfare: At the interface between science and society. *The Veterinary Journal*, 192, 13-19.

OLAGUNJU, L. K., CASPER, D. P. & ANELE, U. Y. 2024. Growth performance of neonatal calves fed milk replacer 2 vs. 3 times per day. *Translational Animal Science*, 8, txae151.

OLLIVETT, T. L., NYDAM, D. V., LINDEN, T. C., BOWMAN, D. D. & VAN AMBURGH, M. E. 2012. Effect of nutritional plane on health and performance in dairy calves after experimental infection with *Cryptosporidium parvum*. *Journal of the American Veterinary Medical Association*, 241, 1514-1520.

OLSON, A., SISCHO, W. M., BERGE, A. C. B., ADAMS-PROGAR, A. & MOORE, D. A. 2019. A retrospective cohort study comparing dairy calf treatment decisions by farm personnel with veterinary observations of clinical signs. *Journal of Dairy Science*, 102, 6391-6403.

ORELLANA RIVAS, R. M., KOMORI, G. H., BEIHLING, V. V., MARINS, T. N., BERNARD, J. K. & TAO, S. 2020. Effects of milk replacer feeding levels on performance and metabolism of preweaned dairy calves during summer. *Journal of Dairy Science*, 103, 313-324.

- OVERVEST, M. A., CROSSLEY, R. E., MILLER-CUSHON, E. K. & DEVRIES, T. J. 2018. Social housing influences the behavior and feed intake of dairy calves during weaning. *Journal of Dairy Science*, 101, 8123-8134.
- PALCZYNSKI, L. J., BLEACH, E. C. L., BRENNAN, M. L. & ROBINSON, P. A. 2020. Appropriate Dairy Calf Feeding from Birth to Weaning: "It's an Investment for the Future". *Animals* [Online], 10.
- PALCZYNSKI, L. J., BLEACH, E. C. L., BRENNAN, M. L. & ROBINSON, P. A. 2022. Youngstock Management as "The Key for Everything"? Perceived Value of Calves and the Role of Calf Performance Monitoring and Advice on Dairy Farms. *Frontiers in Animal Science*, 3.
- PEMPEK, J. A., EASTRIDGE, M. L., SWARTZWELDER, S. S., DANIELS, K. M. & YOHE, T. T. 2016. Housing system may affect behavior and growth performance of Jersey heifer calves. *Journal of Dairy Science*, 99, 569-7
- PENG, R., XIAO, J., CHEN, T., ALUGONGO, G. M., YANG, H., ZHANG, S. & CAO, Z. 2023. Validation of a methodology for characterization of rumination, lying, standing, and performing non-nutritive oral behaviors and behavioral patterns in Holstein dairy calves. *Journal of Dairy Science*, 106, 6402-6415.
- PERTTU, R. K., PEITER, M., BRESOLIN, T., DÓREA, J. R. R. & ENDRES, M. I. 2024. Predictive models for disease detection in group-housed preweaning dairy calves using data collected from automated milk feeders. *Journal of Dairy Science*, 107, 318-328.
- PERTTU, R. K., VENTURA, B. A. & ENDRES, M. I. 2020. Youth and adult public views of dairy calf housing options. *Journal of Dairy Science*, 103, 8507-8517.
- PERTTU, R. K., VENTURA, B. A., RENDAHL, A. K. & ENDRES, M. I. 2021. Public Views of Dairy Calf Welfare and Dairy Consumption Habits of American Youth and Adults. *Frontiers in Veterinary Science*, Volume 8 - 2021.
- PHILIPP, H., SCHMIDT, H., DÜRING, F. & SALAMON, E. 2003. Efficacy of Meloxicam (Metacam®) as Adjunct to a Basic Therapy for the Treatment of Diarrhoea in Calves. *Acta Veterinaria Scandinavica*, 44, P95.
- PLAUGHER, G. D. & CANTOR, M. C. 2025. A scoping review on pair housing dairy calves: health and performance outcomes and tactics to reduce cross-sucking behavior. *Frontiers in Veterinary Science*, Volume 12 - 2025.
- PROKOP, L., HOFFMANN, G., KASKE, M. & WIEDEMANN, S. 2025. Effect of an Ad Libitum Milk Supply During the First Three Weeks of Life of Dairy Calves on Heart Rate and Heart Rate Variability During Feeding and Rehousing. *Veterinary Sciences* [Online], 12.
- PROUDFOOT, K. L., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2012. Linking the social environment to illness in farm animals. *Applied Animal Behaviour Science*, 138, 203-215.
- RAMOS, J. S., VILLETAZ ROBICHAUD, M., DUBUC, J., SANTSCHI, D., ROY, J.-P., FECTEAU, G. & BUCZINSKI, S. 2025. The burden of disease and syndromes in preweaning dairy

calves at the farm level: Estimation based on the disability-adjusted life years (DALY) approach. *Journal of Dairy Science*, 108, 11376-11388.

RICHTER, S. H., KÄSTNER, N., KRIWET, M., KAISER, S. & SACHSER, N. 2016. Play matters: the surprising relationship between juvenile playfulness and anxiety in later life. *Animal Behaviour*, 114, 261-271.

RIESGRAF, K. A., WEIGEL, K. A., AKINS, M. S. & VAN OS, J. M. C. 2024. Long-Term Effects of Pre-Weaning Individual or Pair Housing of Dairy Heifer Calves on Subsequent Growth and Feed Efficiency. *Animals* [Online], 14.

RILEY, B., HASKELL, M., DUTHIE, C. A., MASON, C. & CORBISHLEY, A. 2024a. Saliva pH as a diagnostic indicator of neonatal calf diarrhoea. *Cattle Practice*, 32, 110.

RILEY, B. B., CORBISHLEY, A., HASKELL, M. J., DUTHIE, C.-A., MACRAE, A. I., BURROUGH, E. & MASON, C. 2024b. Salivary pH, but not conductivity, is an indicator of diarrhea in neonatal calves. *Frontiers in Veterinary Science*, 11.

RILEY, B. B., DUTHIE, C.-A., CORBISHLEY, A., MASON, C., BOWEN, J. M., BELL, D. J. & HASKELL, M. J. 2023. Intrinsic calf factors associated with the behavior of healthy pre-weaned group-housed dairy-bred calves. *Frontiers in Veterinary Science*, 10, 1204580.

RITTER, C., MILLS, K. E., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2020. Perspectives of western Canadian dairy farmers on the future of farming. *Journal of Dairy Science*, 103, 10273-10282.

ROCHE, S., RENAUD, D. L., BAUMAN, C. A., LOMBARD, J., SHORT, D., SARACENI, J. & KELTON, D. F. 2023. Calf management and welfare in the Canadian and US dairy industries: Where do we go from here? *Journal of Dairy Science*, 106, 4266-4274.

ROCHE, S. M., GENORE, R., RENAUD, D. L., SHOCK, D. A., BAUMAN, C., CROYLE, S., BARKEMA, H. W., DUBUC, J., KEEFE, G. P. & KELTON, D. F. 2020. Short communication: Describing mortality and euthanasia practices on Canadian dairy farms. *Journal of Dairy Science*, 103, 3599-3605.

ROSE, D. C. & CHILVERS, J. 2018. Agriculture 4.0: Broadening Responsible Innovation in an Era of Smart Farming. *Frontiers in Sustainable Food Systems*, Volume 2 - 2018.

ROSE, D. C., MORRIS, C., LOBLEY, M., WINTER, M., SUTHERLAND, W. J. & DICKS, L. V. 2018. Exploring the spatialities of technological and user re-scripting: The case of decision support tools in UK agriculture. *Geoforum*, 89, 11-18.

ROSE, D. C., SUTHERLAND, W. J., PARKER, C., LOBLEY, M., WINTER, M., MORRIS, C., TWINING, S., FFOULKES, C., AMANO, T. & DICKS, L. V. 2016. Decision support tools for agriculture: Towards effective design and delivery. *Agricultural Systems*, 149, 165-174.

ROSENBERGER, K., COSTA, J. H. C., NEAVE, H. W., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2017. The effect of milk allowance on behavior and weight gains in dairy calves. *Journal of Dairy Science*, 100, 504-512.

RUMINANT HEALTH & WELFARE GROUP 2025. GB Dairy Calf Strategy: A review of progress 2020-2023. <https://ruminanthw.org.uk/wp-content/uploads/2025/01/GB-dairy-calf-strategy-review-of-progress-Jan-2025-APP-compressed.pdf>

RUSHEN, J. & DE PASSILLÉ, A. M. 2012. Automated measurement of acceleration can detect effects of age, dehorning and weaning on locomotor play of calves. *Applied Animal Behaviour Science*, 139, 169-174.

RUSSELL, E. R., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2025. Milk feeding and calf housing practices on British Columbia dairy farms. *Journal of Dairy Science*, 108, 2875-2882.

SALTER, R. S., REUSCHER, K. J. & VAN OS, J. M. C. 2021. Milk- and starter-feeding strategies to reduce cross sucking in pair-housed calves in outdoor hutches. *Journal of Dairy Science*, 104, 6096-6112.

SANTMAN-BERENDS, I., SCHUKKEN, Y. H. & VAN SCHAİK, G. 2019. Quantifying calf mortality on dairy farms: Challenges and solutions. *Journal of Dairy Science*, 102, 6404-6417.

SELVAGGI, R., PAPPALARDO, G., ZARBÀ, C. & LUSK, J. L. 2024. Driving factors behind precision livestock farming tools adoption: The case of the pedometer on dairy farms. *Agricultural Systems*, 220, 104090.

SHARON, K. P., LIANG, Y., SANCHEZ, N. C. B., CARROLL, J. A., BROADWAY, P. R., DAVIS, E. M. & BALLOU, M. A. 2019. Pre-weaning plane of nutrition and Mannheimia haemolytica dose influence inflammatory responses to a bovine herpesvirus-1 and Mannheimia haemolytica challenge in post-weaning Holstein calves. *Journal of Dairy Science*, 102, 9082-9096.

SIACHOS, N., GRIFFITHS, B. E., WILSON, J. P., BEDFORD, C., ANAGNOSTOPOULOS, A., NEARY, J. M., SMITH, R. F. & OIKONOMOU, G. 2025. Evaluation of a fully automated 2-dimensional imaging system for real-time cattle lameness detection using machine learning. *Journal of Dairy Science*, 108, 4206-4224.

SIROVICA, L. V., RITTER, C., HENDRICKS, J., WEARY, D. M., GULATI, S. & VON KEYSERLINGK, M. A. G. 2022. Public attitude toward and perceptions of dairy cattle welfare in cow-calf management systems differing in type of social and maternal contact. *Journal of Dairy Science*, 105, 3248-3268.

SINNOTT, A. M., KENNEDY, E. & BOKKERS, E. A. M. 2021. The effects of manual and automated milk feeding methods on group-housed calf health, behaviour, growth and labour. *Livestock Science*, 244.

SISCHO, W. M., MOORE, D. A., PEREIRA, R., WARNICK, L., MOORE, D. L., VANEGAS, J., KURTZ, S., HEATON, K., KINDER, D., SILER, J. & DAVIS, M. A. 2019. Calf care personnel on dairy farms and their educational opportunities. *Journal of Dairy Science*, 102, 3501-3511.

- SMITH, A.-L., DOIDGE, C. & KALER, J. 2025. Comparing expert stakeholders' assessments of the relative impact of management practices on dairy calf welfare using best-worst scaling. *Preventive Veterinary Medicine*, 242, 106587.
- SOBERON, F. & VAN AMBURGH, M. E. 2013. Lactation Biology Symposium: The effect of nutrient intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as adults: a meta-analysis of current data. *Journal of Animal Science*, 91, 706-12.
- ŠOLTÉSOVÁ, H., NAGY, O., TÓTHOVÁ, C., PAULÍKOVÁ, I. & SEIDEL, H. 2015. Blood Gases, Acid-Base Status And Plasma Lactate Concentrations In Calves With Respiratory Diseases. *Acta Veterinaria*, 65, 111-124.
- SØRENSEN, C. G., FOUNTAS, S., NASH, E., PESONEN, L., BOCHTIS, D., PEDERSEN, S. M., BASSO, B. & BLACKMORE, S. B. 2010. Conceptual model of a future farm management information system. *Computers and Electronics in Agriculture*, 72, 37-47.
- STACHOWICZ, J. & UMSTÄTTER, C. 2021. Do we automatically detect health-or general welfare-related issues? A framework. *Proceedings of the Royal Society B*, 288, 20210190.
- STEENSELS, M., ANTLER, A., BAHR, C., BERCKMANS, D., MALTZ, E. & HALACHMI, I. 2016. A decision-tree model to detect post-calving diseases based on rumination, activity, milk yield, BW and voluntary visits to the milking robot. *Animal*, 10, 1493-500.
- STYGAR, A. H., GÓMEZ, Y., BERTESELLI, G. V., DALLA COSTA, E., CANALI, E., NIEMI, J. K., LLONCH, P. & PASTELL, M. 2021. A Systematic Review on Commercially Available and Validated Sensor Technologies for Welfare Assessment of Dairy Cattle. *Frontiers in Veterinary Science*, Volume 8 - 2021.
- SUMNER, C. L. & VON KEYSERLINGK, M. A. G. 2018. Canadian dairy cattle veterinarian perspectives on calf welfare. *Journal of Dairy Science*, 101, 10303-10316.
- SUMNER, C. L., VON KEYSERLINGK, M. A. G. & WEARY, D. M. 2018. How benchmarking motivates farmers to improve dairy calf management. *Journal of Dairy Science*, 101, 3323-3333.
- SUN, D., WEBB, L., VAN DER TOL, P. P. J. & VAN REENEN, K. 2021. A Systematic Review of Automatic Health Monitoring in Calves: Glimpsing the Future From Current Practice. *Frontiers in Veterinary Science*, 8, 761468.
- SUTHERLAND, M. A., LOWE, G. L., HUDDART, F. J., WAAS, J. R. & STEWART, M. 2018. Measurement of dairy calf behavior prior to onset of clinical disease and in response to disbudding using automated calf feeders and accelerometers. *Journal of Dairy Science*, 101, 8208-8216.
- SUTHERLAND, M. A., WORTH, G. M., SCHÜTZ, K. E. & STEWART, M. 2014. Rearing substrate and space allowance influences locomotor play behaviour of dairy calves in an arena test. *Applied Animal Behaviour Science*, 154, 8-14.

- SVENSSON, C., HEGRESTAD, A. L. & LINDBLOM, J. 2023. Dairy farmer and farm staff attitudes and perceptions regarding daily milk allowance to calves. *Journal of Dairy Science*, 106, 7220-7239.
- SVENSSON, C. & JENSEN, M. B. 2007. Short Communication: Identification of Diseased Calves by Use of Data from Automatic Milk Feeders. *Journal of Dairy Science*, 90, 994-997.
- SVENSSON, C. & LIBERG, P. 2006. The effect of group size on health and growth rate of Swedish dairy calves housed in pens with automatic milk-feeders. *Preventive Veterinary Medicine*, 73, 43-53.
- SVENSSON, C., LINDER, A. & OLSSON, S. O. 2006. Mortality in Swedish Dairy Calves and Replacement Heifers. *Journal of Dairy Science*, 89, 4769-4777.
- SVENSSON, C., LUNDBORG, K., EMANUELSON, U. & OLSSON, S.-O. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Preventive Veterinary Medicine*, 58, 179-197.
- SWARTZ, T. H., FINDLAY, A. N. & PETERSSON-WOLFE, C. S. 2017. Short communication: Automated detection of behavioral changes from respiratory disease in pre-weaned calves. *Journal of Dairy Science*, 100, 9273-9278.
- SYLVIA, K. E. & DEMAS, G. E. 2017. A Return to Wisdom: Using Sickness Behaviors to Integrate Ecological and Translational Research. *Integrative and Comparative Biology*, 57, 1204-1213.
- THEURER, M. E., WHITE, B. J., LARSON, R. L. & SCHROEDER, T. C. 2015. A stochastic model to determine the economic value of changing diagnostic test characteristics for identification of cattle for treatment of bovine respiratory disease. *Journal of Animal Science*, 93, 1398-410.
- THIBAUT, M., PAILLER, S. & FREUND, D. 2022. Why Are They Buying It?: United States Consumers' Intentions When Purchasing Meat, Eggs, and Dairy With Welfare-related Labels. *Food Ethics*, 7.
- THOMAS, M., OCCHIUTO, F., VÁZQUEZ-DIOSDADO, J. A. & KALER, J. 2025. Diurnality is consistently different between individuals and decreases with disease or stressful events in dairy calves. *Scientific Reports*, 15, 27090.
- TOAFF-ROSENSTEIN, R. L. & TUCKER, C. B. 2018. The sickness response at and before clinical diagnosis of spontaneous bovine respiratory disease. *Applied Animal Behaviour Science*, 201, 85-92.
- TODD, C. G., MILLMAN, S. T., MCKNIGHT, D. R., DUFFIELD, T. F. & LESLIE, K. E. 2010. Nonsteroidal anti-inflammatory drug therapy for neonatal calf diarrhea complex: Effects on calf performance. *Journal of Animal Science*, 88, 2019-2028.

- TUYTTENS, F. A. M., MOLENTO, C. F. M. & BENAÏSSA, S. 2022. Twelve Threats of Precision Livestock Farming (PLF) for Animal Welfare. *Frontiers in Veterinary Science*, Volume 9 - 2022.
- UMAÑA SEDÓ, S. G., WINDER, C. B., PERRY, K. V., CASWELL, J. L., MEE, J. F. & RENAUD, D. L. 2024. Herd-level risk factors associated with preweaning mortality on Ontario dairy farms. *Journal of Dairy Science*, 107, 11502-11512.
- UMAÑA SEDÓ, S. G., WINDER, C. B. & RENAUD, D. L. 2023. Graduate Student Literature Review: The problem of calf mortality on dairy farms. *Journal of Dairy Science*, 106, 7164-7176.
- URIE, N. J., LOMBARD, J. E., SHIVLEY, C. B., KOPRAL, C. A., ADAMS, A. E., EARLEYWINE, T. J., OLSON, J. D. & GARRY, F. B. 2018a. Preweaned heifer management on US dairy operations: Part I. Descriptive characteristics of preweaned heifer raising practices. *Journal of Dairy Science*, 101, 9168-9184.
- URIE, N. J., LOMBARD, J. E., SHIVLEY, C. B., KOPRAL, C. A., ADAMS, A. E., EARLEYWINE, T. J., OLSON, J. D. & GARRY, F. B. 2018b. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. *Journal of Dairy Science*, 101, 9229-9244.
- VAN DE GUCHT, T., SAEYS, W., VAN NUFFEL, A., PLUYM, L., PICCART, K., LAUWERS, L., VANGEYTE, J. & VAN WEYENBERG, S. 2017. Farmers' preferences for automatic lameness-detection systems in dairy cattle. *Journal of Dairy Science*, 100, 5746-5757.
- VAN DER BURG, S., BOGAARDT, M.-J. & WOLFERT, S. 2019. Ethics of smart farming: Current questions and directions for responsible innovation towards the future. *NJAS: Wageningen Journal of Life Sciences*, 90-91, 1-10.
- VAN STAAVEREN, N., HYLAND, E., HOULAHAN, K., LYNCH, C., MIGLIOR, F., KELTON, D. F., SCHENKEL, F. S. & BAES, C. F. 2023. Recording of calf diseases for potential use in breeding programs: a case study on calf respiratory illness and diarrhea. *Canadian Journal of Animal Science*, 103, 192-203.
- VASSEUR, E., RUSHEN, J., DE PASSILLÉ, A. M., LEFEBVRE, D. & PELLERIN, D. 2010. An advisory tool to improve management practices affecting calf and heifer welfare on dairy farms. *Journal of Dairy Science*, 93, 4414-4426.
- VEISSIER, I., MIALON, M.-M. & SLOTH, K. H. 2017. Short communication: Early modification of the circadian organization of cow activity in relation to disease or estrus. *Journal of Dairy Science*, 100, 3969-3974.
- VON KEYSERLINGK, M. A. G., RUSHEN, J., DE PASSILLÉ, A. M. & WEARY, D. M. 2009. Invited review: The welfare of dairy cattle: Key concepts and the role of science. *Journal of Dairy Science*, 92, 4101-4111.

- WALKER, M. D., DUGGAN, G., ROULSTON, N., VAN SLACK, A. & MASON, G. 2012. Negative affective states and their effects on morbidity, mortality and longevity. *Animal Welfare*, 21, 497-509.
- WEARY, D. M., HUZZEY, J. M. & VON KEYSERLINGK, M. A. 2009. Board-invited review: Using behavior to predict and identify ill health in animals. *Journal of Animal Science*, 87, 770-7.
- WELK, A., CANTOR, M. C., NEAVE, H. W., COSTA, J. H. C., MORRISON, J. L., WINDER, C. B. & RENAUD, D. L. 2025. Effect of nonsteroidal anti-inflammatory drugs on neonatal calf diarrhea when administered at a disease alert generated by automated milk feeders. *Journal of Dairy Science*, 108, 1842-1854.
- WELK, A., OTTEN, N. D. & JENSEN, M. B. 2023. Invited review: The effect of milk feeding practices on dairy calf behavior, health, and performance: A systematic review. *Journal of Dairy Science*, 106, 5853-5879.
- WHALIN, L., BARTH, K., BERTELSEN, M., BOKKERS, E. A. M., FERNEBORG, S., HASKELL, M. J., IVEMEYER, S., JENSEN, M. B., JOHANSEN, J. R. E., MEJDELL, C. M., MUGHAL, M., NEAVE, H. W., VAARST, M., VAN KNEGSEL, A., VAN ZYL, C. L., WEGNER, C. S. & JOHNSON, J. F. 2025. Invited review: Future directions for cow-calf contact research and sustainable on-farm applications. *Journal of Dairy Science*, 108, 6550-6564.
- WHALIN, L., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2018. Short communication: Pair housing dairy calves in modified calf hutches. *Journal of Dairy Science*, 101, 5428-5433.
- WHALIN, L., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2021. Understanding Behavioural Development of Calves in Natural Settings to Inform Calf Management. *Animals*, 11.
- WHALIN, L., WEARY, D. M. & VON KEYSERLINGK, M. A. G. 2022. Prewaning dairy calves' preferences for outdoor access. *Journal of Dairy Science*, 105, 2521-2530.
- WHITE, B. J., GOEHL, D. R., AMRINE, D. E., BOOKER, C., WILDMAN, B. & PERRETT, T. 2016. Bayesian evaluation of clinical diagnostic test characteristics of visual observations and remote monitoring to diagnose bovine respiratory disease in beef calves. *Preventative Veterinary Medicine*, 126, 74-80.
- WILMS, J. N., GHAFARI, M. H., STEELE, M. A., SAUERWEIN, H., MARTÍN-TERESO, J. & LEAL, L. N. 2022. Macronutrient profile in milk replacer or a whole milk powder modulates growth performance, feeding behavior, and blood metabolites in ad libitum-fed calves. *Journal of Dairy Science*, 105, 6670-6692.
- WILSON, D. J., HABING, G., WINDER, C. B. & RENAUD, D. L. 2023a. A scoping review of neonatal calf diarrhea case definitions. *Preventive Veterinary Medicine*, 211, 105818.
- WILSON, D. J., PEMPEK, J. A., ROCHE, S. M., CREUTZINGER, K. C., LOCKE, S. R., HABING, G., PROUDFOOT, K. L., GEORGE, K. A. & RENAUD, D. L. 2021. A focus group study of

Ontario dairy producer perspectives on neonatal care of male and female calves. *Journal of Dairy Science*, 104, 6080-6095.

WILSON, D. J., ROCHE, S. M., PEMPEK, J. A., HABING, G., PROUDFOOT, K. L. & RENAUD, D. L. 2023b. How benchmarking motivates colostrum management practices on dairy farms: A realistic evaluation. *Journal of Dairy Science*, 106, 9200-9215.

WILSON, D. J., SARACENI, J., ROCHE, S. M., PEMPEK, J. A., HABING, G., PROUDFOOT, K. L. & RENAUD, D. L. 2024. How can better calf care be realized on dairy farms? A qualitative interview study of veterinarians and farmers. *Journal of Dairy Science*, 107, 1694-1706.

WOLFERT, S., GE, L., VERDOUW, C. & BOGAARDT, M.-J. 2017. Big Data in Smart Farming – A review. *Agricultural Systems*, 153, 69-80.

WOOD-GUSH, D. G. M. & VESTERGAARD, K. 1989. Exploratory behavior and the welfare of intensively kept animals. *Journal of Agricultural Ethics*, 2, 161-169.

Appendices

Appendix 1 Supplementary material for Chapter 2

Table A1.1 Sire breed type, sub-herd, and sex of the thirty-one calves included in the analysis.

Dairy or beef	Sub-herd	Sex	
		Male	Female
Beef	A	0	1
	B	5	5
Dairy	A	17	0
	B	0	3

Table A1.2 Correlation coefficients of three explanatory variables in thirty-one healthy pre-weaned calves

	Age	Live-weight	Birthweight
Age	-	0.56	-0.04
Live weight	0.56	-	0.54
Birth weight	-0.04	0.54	-

Table A1.3 Descriptive statistics of possible explanatory variables in thirty-one healthy pre-weaned calves

	Mean	Standard deviation	Minimum	Maximum	Standard error of the mean
Age (days)	27.7	6.0	11	39	0.3
Live Weight (Kg)	56.5	7.2	41.9	79.9	0.4
Birth weight (Kg)	43.6	5.2	32.0	52.0	1.0
Age at inclusion into the group pen (days)	8.7	2.0	6	13	0.4

Table A1.4 Descriptive statistics of feeding and activity behaviours in thirty-one healthy pre-weaned calves

	Mean	Standard deviation	Minimum	Maximum	Standard error of the mean
Daily lying time (minutes)	973.7	63.7	679.2	1161.6	3.7
Daily standing time (minutes)	346.2	63.8	158.4	640.8	3.7
Daily lying bouts (n)	19.3	5.7	8.0	35.0	0.3
Daily standing bouts (n)	19.3	5.8	7.0	35.0	0.3
Total daily motion index	3328.5	1459.4	454.0	7445.0	83.8
Mean lying bout length (minutes)	55.3	18.2	22.4	139.3	1.0
Mean standing bout length (minutes)	19.6	7.3	5.6	54.4	0.4
Mean motion index per standing bout	177.1	75.6	32.4	470.1	4.4
Total time at milk (minutes)	15.8	3.5	4.5	35.6	0.2
Total milk visits (n)	8.5	2.2	2.0	16.0	0.1
Mean milk visit length (minutes)	1.8	0.6	1.1	4.3	0.0
Mean milk drinking speed (g/s)	6.8	1.4	1.0	10.4	0.1
Daily milk volume (ml)	6639.9	1012.9	1746.00	8416.0	57.5
Mean milk per feed (ml)	770.7	216.2	229.5	1380.8	12.3

Table A1.5 Final linear mixed effect model outputs showing factors affecting live-weight and birthweight in thirty healthy pre-weaned calves. Factors with $p < 0.05$ are shown in bold.

	Fixed effect	Level	Effect size	Confidence interval	P value
Live weight	Dairy or beef	Beef	Reference	Reference	Reference
		Dairy	-3.5	-6.5- -0.5	0.032
	Age		0.8	0.8-0.8	< 0.001
	Birth weight		0.8	0.5- 1.1	< 0.001
Birthweight	Sex	Female	Reference	Reference	Reference
		Male	3.6	0.3- 6.9	0.050
	Dam Lactation	1	Reference	Reference	Reference
		2	-7.2	1.5- 8.6	0.013
		3+	-6.7	2.9- 9.7	0.002

Table A1.6 Correlation coefficients of milk drinking behaviors in thirty-one healthy pre-weaned calves

	Total time at milk	Total milk visits	Mean milk visit length	Mean milk drinking speed	Daily milk volume	Mean milk per feed
Total time at milk	-	0.52	0.22	-0.64	0.11	-0.38
Total milk visits	0.52	-	-0.54	-0.09	0.39	-0.70
Mean milk visit length	0.22	-0.54	-	-0.51	-0.50	0.57
Mean milk drinking speed	-0.64	-0.09	-0.51	-	0.54	0.32
Daily milk volume	0.11	0.39	-0.50	0.54	-	0.06
Mean milk per feed	-0.38	-0.70	0.57	0.32	0.06	-

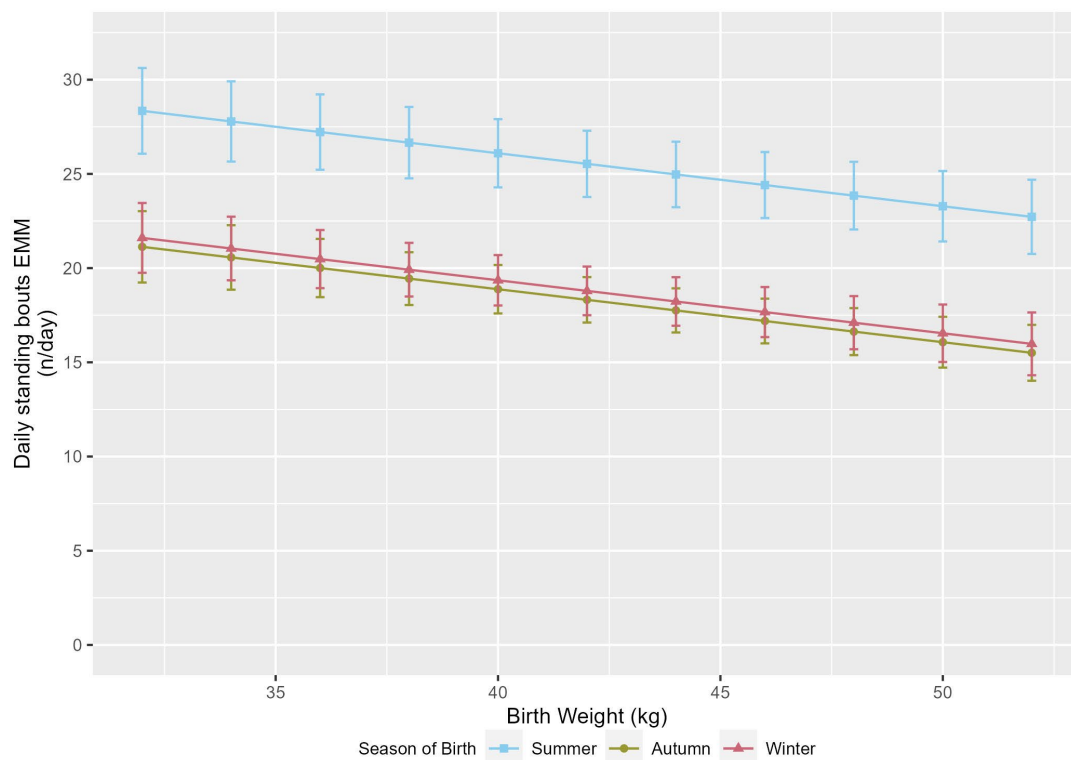


Figure A1.1 The estimated means of daily standing bouts in relation to season and birthweight. Error bars denote the standard error.

Appendix 2 Supplementary material for Chapter 4

Table A2.1 Samples partitioned by disease status, sire breed type and sex.

Sex	Sire breed type			
	Beef		Dairy	
	Female	Male	Female	Male
Healthy	72	66	18	8
NCD-H	119	129	45	21
NCD-D	3	4	3	

Table A2.2 Descriptive statistics of calf variables, saliva parameters, hematocrit and plasma total protein partitioned by disease status at the time of sample collection

Variable	Disease status	Mean	SD	Median	Minimum	Maximum	SEM
Birthweight (kg)	Healthy	44.08	5.93	43.5	29	63	0.47
	NCD-H	42.09	5.82	42	28	56.5	0.34
	NCD-D	41.5	5.72	40	36	54	1.91
Age at inclusion (days)	Healthy	7.27	0.74	7	6	11	0.06
	NCD-H	7.11	0.44	7	6	9	0.02
	NCD-D	7	0	7	7	7	0
Age (days)	Healthy	12.13	5.19	9	7	25	0.4
	NCD-H	14.67	3.55	15	8	23	0.2
	NCD-D	14.6	3.57	15	8	20	1.13
Saliva conductivity (mS/cm)	Healthy	7.06	1.67	6.8	3.55	12.99	0.13
	NCD-H	7.31	1.61	7.16	3.75	11.65	0.09
	NCD-D	6.67	0.9	6.85	4.77	7.72	0.28
Saliva pH	Healthy	8.17	0.23	8.18	7.41	8.76	0.02
	NCD-H	8.1	0.24	8.12	7.1	8.76	0.01
	NCD-D	7.89	0.31	7.94	7.34	8.22	0.1
Hematocrit (%)	Healthy	40.44	5.63	40	25	58	0.44
	NCD-H	40.07	6.35	40	24	59	0.36
	NCD-D	44.4	5.41	44.5	37	52.5	1.71
Plasma total protein (g/dL)	Healthy	6.1	0.65	6.07	4.5	8.1	0.05
	NCD-H	5.76	0.55	5.75	4.25	7.35	0.03
	NCD-D	5.94	0.6	5.82	5.15	7.2	0.19

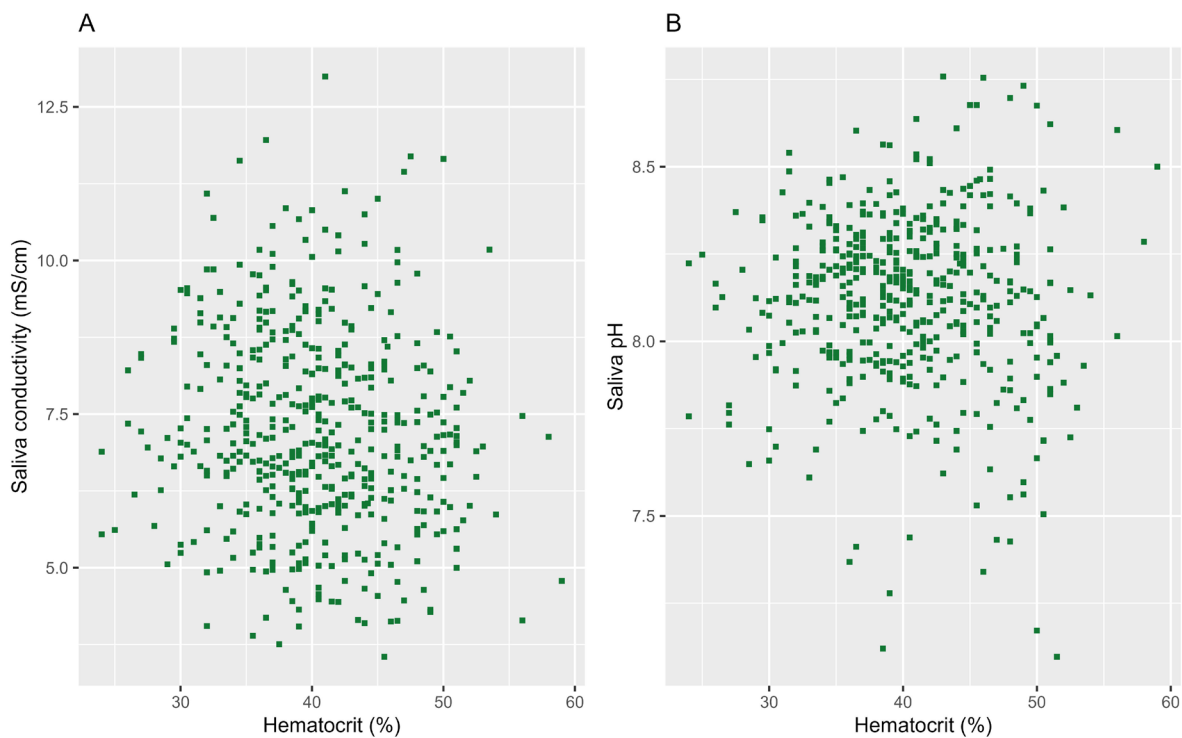


Figure A2.1 Scatterplots showing the relationships between (A) saliva pH and hematocrit and (B) saliva conductivity and hematocrit

Table A2.3 Descriptive statistics of the calf variables partitioned by disease status in a balanced data set of 30 calf days

Variable	Disease status	Mean	SD	Median	Minimum	Maximum	SEM
Birthweight (kg)	Healthy	42.15	5.94	42.5	33.5	51	1.88
	NCD-H	39.7	3.95	41	32	44.5	1.25
	NCD-D	41.5	5.72	40	36	54	1.91
Age at inclusion (days)	Healthy	7.4	0.7	7	7	9	0.22
	NCD-H	7.1	0.74	7	6	9	0.23
	NCD-D	7	0	7	7	7	0
Age (days)	Healthy	12.5	5.72	9	8	22	1.81
	NCD-H	14.9	3.6	15.5	10	19	1.14
	NCD-D	14.6	3.57	15	8	20	1.13

Table A2.4 Descriptive statistics of the serum biochemistry parameters partitioned by disease status in a balanced data set of 30 calf days

Variable	Disease status	Mean	SD	Median	Minimum	Maximum	SEM
Serum total protein (g/L)	Healthy	60.54	7.26	59.2	50.2	73.4	2.3
	NCD-H	60	8.79	59.3	43	73.5	2.78
	NCD-D	58.52	4.38	57.15	53.6	66.2	1.39
Albumin (g/L)	Healthy	26.11	2.01	26.7	22.7	28.2	0.64
	NCD-H	26.32	1.77	26	24	29.8	0.56
	NCD-D	29.62	2.72	28.35	27	34.4	0.86
Globulin (g/L)	Healthy	58.52	4.38	57.15	53.6	66.2	1.39
	NCD-H	34.43	7.91	32.95	23.8	50.4	2.5
	NCD-D	33.68	9.53	32.95	15.4	49	3.01
Sodium (mmol/L)	Healthy	98.3	1.42	98	95	100	0.45
	NCD-H	97.6	2.5	97	94	102	0.79
	NCD-D	99	2.75	98.5	95	103	0.87
Potassium (mmol/L)	Healthy	5.21	0.52	5.15	4.4	6	0.17
	NCD-H	4.99	0.31	5	4.5	5.4	0.1
	NCD-D	5.24	0.47	5.1	4.9	6.5	0.15
Chloride (mmol/L)	Healthy	98.3	1.42	98	95	100	0.45
	NCD-H	97.6	2.5	97	94	102	0.79
	NCD-D	99	2.75	98.5	95	103	0.87
Strong ion difference (mmol/L)	Healthy	49.01	3	48.25	46.2	56.8	0.95
	NCD-H	48.69	3.58	48.15	44.8	56.3	1.13
	NCD-D	42.64	3.53	42.8	37.3	46.9	1.12

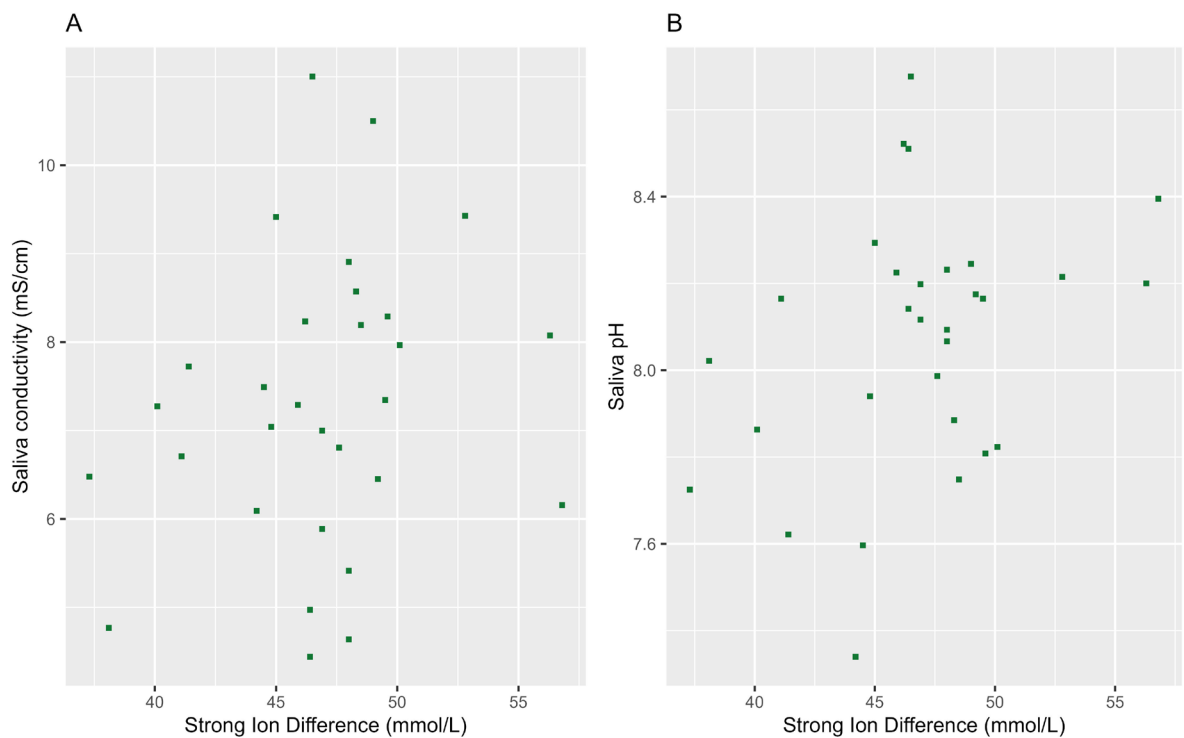


Figure A2.2 Scatterplots showing the relationships between strong ion difference and (A) saliva conductivity, (B) saliva pH in a balanced dataset of 30 calf days.

A2.1 Calf factors associated with differences in saliva and blood parameters in healthy calves.

A2.1.1 Methods

The data from the healthy samples was analyzed to check for any known calf factors that may be associated with altered saliva and blood parameters. Univariable general linear mixed models were constructed for hematocrit, PTP, saliva conductivity and saliva pH in turn. The calf identity nested within group was used as a random effect in all models. Age, date of sampling, sire breed-type (beef or dairy), sex, and birthweight were each tested as fixed effects in a univariable model constructed using `lmer()` in the `lme4` (Bates *et al.*, 2015) package of R. The model results were checked using `summary()` in the `lmerTest` package (Kuznetsova *et al.*, 2017). Biologically plausible interactions were checked at this stage. Those fixed effects where $p < 0.2$ and interactions where $p < 0.05$ were taken forward for multivariable general linear mixed model building. A forward model building approach was used, adding variables in order of their p values starting with the lowest. The final model was chosen using the corrected Akaike Information Criterion calculated using `aictab()` (AICcmodavg, Mazerolle, 2020). The final models were checked using the `simulateResiduals()` and `plotQqunif()` functions in the `DHARMA` package (Hartig, 2022). Estimated and p values were calculated using the `summary()` function in the `lmerTest` package (Kuznetsova *et al.*, 2017). The confidence intervals were calculated using the `confint()` function (R Core Team, 2021). The numerator degrees of freedom (NDF) and denominator degrees of freedom (DDF) were calculated using the `anova()` function (R Core Team, 2021).

A2.1.2 Results

One hundred and sixty-four healthy sampling events from 104 calves were available for analysis. The number of samples within each sex and sire breed-type is summarized in Table A2.1. Descriptive statistics of the healthy sampling events are summarized in Table A2.2 of the supplementary materials. The results of the mixed models for saliva conductivity, saliva pH, hematocrit and PTP are shown in Table A2.5.

Saliva conductivity was higher in male calves when compared to their female counterparts ($p = 0.04$, Table A2.5). Date was included in the final model as it improved model fit ($p = 0.054$, Table A2.5).

Saliva pH had a significant interaction between age and sex ($p < 0.05$, Table A2.5, Figure A2.3). However, neither age or sex were significant individually ($p = 0.20$, and $p = 0.17$, respectively, Table A2.5).

Hematocrit increased over the course of the study period ($p = 0.03$, $t = 2.678$, NDF = 1, DDF = 7.56, Table A2.5). Male calves had a lower hematocrit than their female counterparts ($p = 0.0037$, $t = -2.978$, NDF = 1, DDF = 93.624, Table A2.5). Dairy-sired calves had a lower hematocrit than their beef-sired counterparts ($p = 0.0215$, $t = -2.333$, NDF = 1, DDF = 110.358, Table A2.5).

PTP declined with age ($p < 0.0001$, $t = 5-.234$, NDF = 1, DDF = 81.998, Table A2.5). Calves with a dairy sire had a lower PTP than those with a beef sire ($p = 0.0355$, $t = -2.129$, NDF = 1, DDF = 108.499, Table A2.5).

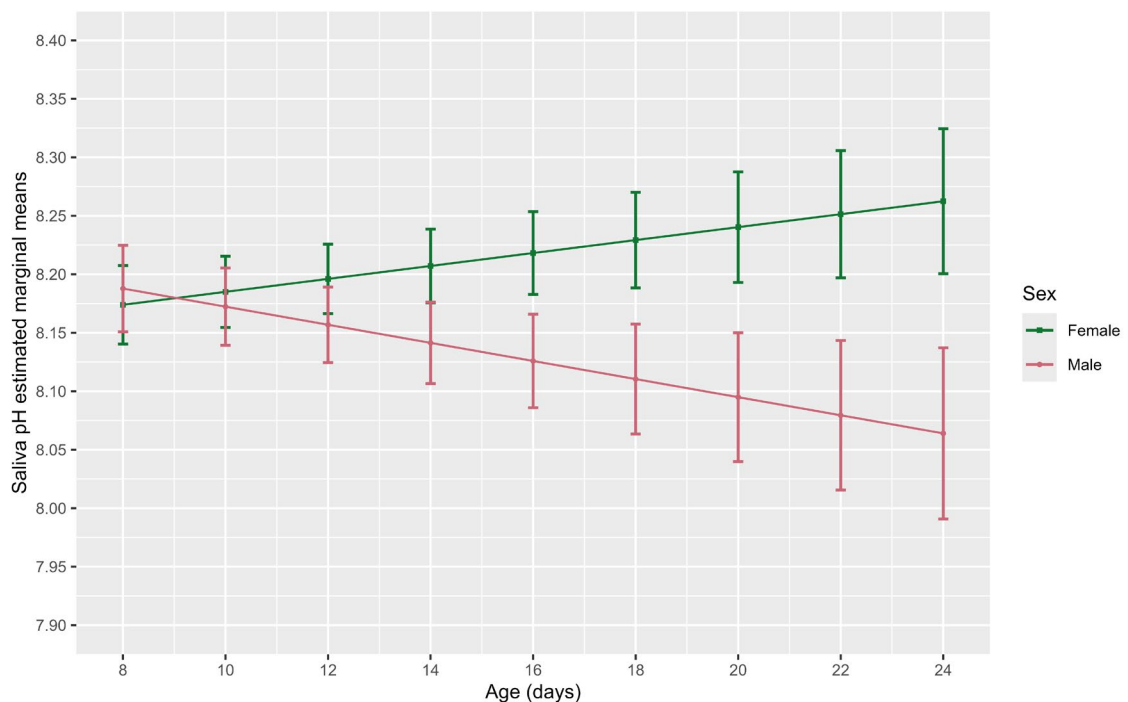


Figure A2.3 Association of sex and age with saliva pH. Plots and error bars denote the estimated marginal means (EMM) and corresponding standard errors calculated from the final model. Differing letters indicate statistically significant differences

Table A2.5 Variables associated with differences in saliva and blood parameters in healthy artificially reared calves. The results shown are outputs from the final linear mixed models. Variables shown in bold have $p < 0.05$.

Variable	Factor	Level	Number of calf days	Estimate	Confidence interval	Numerator degrees of freedom	Denominator degrees of freedom	T value	P value
Saliva Conductivity (mS/cm)	Date		164 ^a	-0.01	-0.01 - -0.00	1	10.644	-2.171	0.054
	Sex	Female	90 ^b	Reference	Reference	1	97.731	Reference	Reference
		Male	74^c	0.59	0.04 - 1.15			2.100	0.038
Saliva pH	Age		164 ^a	0.01	-0.00 - -0.01	1	140.93	1.287	0.200
	Sex	Female	90 ^b	Reference	Reference	1	139.65	Reference	Reference
		Male	74 ^c	0.12	-0.05 - 0.29			1.388	0.742
		Age*Sex				1	140.32	-1.986	0.049
Hematocrit (%)	Date		164^a	0.02	0.01 - 0.04	1	6.5361	3.237	0.030
	Sex	Female	90 ^b	Reference	Reference	1	94.253	Reference	Reference
		Male	74^c	-2.75	-4.50 - -0.82			-2.952	0.004
	Sire breed-type	Beef	138 ^d	Reference	Reference	1	115.270	Reference	Reference
	Dairy	26^e	-2.85	-5.25 -0.48			-2.406	0.022	
Plasma Total Protein (g/dL)	Age		164^a	-0.03	-0.05 - -0.02	1	82.067	-5.356	< 0.001
	Sire breed-type	Beef	138 ^d	Reference	Reference	1	111.730	Reference	Reference
	Dairy	26^e	-0.32	-0.62 - -0.03			-2.163	0.03	

^a104 calves, ^b57 calves, ^c48 calves, ^d85 calves, ^e20 calves

A2.1.3 Bibliography

BATES, D., MÄCHLER, M., BOLKER, B. & WALKER, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67, 1-48.

HARTIG, F. 2022. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. <https://CRAN.R-project.org/package=DHARMA>.

KUZNETSOVA, A., BROCKHOFF, P. B. & CHRISTENSEN, R. H. B. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1-26.

MAZEROLLE, M. 2020. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). <https://cran.r-project.org/package=AICcmodavg>.

R CORE TEAM 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.