Statistical learning approaches to optimise the health, welfare, and productivity of calves on dairy farms

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Abstract

The effective management of preweaned calves is one of the most important areas of dairy farm management, and can have substantial impacts in terms of health, welfare, and productivity. It is critical that veterinary advisors are able to implement proactive changes to housing and management likely to not only result in the largest improvements, but also be applicable to the majority of farms. The majority of statistical techniques are intended for a relatively low dimensional setting, and conventional statistical approaches are often poorly suited to problems where the number of variables exceed the number of observations. The number of potential variables available in today's data rich environment is increasing, and the robust identification of causal variables is becoming increasingly important. Statistical learning techniques represent important tools in identifying factors associated with calf health and performance on dairy farms.

In Chapter 2, over 21 million cattle deaths were analysed utilising national birth and death registrations from the national British Cattle Movement Service, to quantify the temporal incidence rate, distributional features, and factors affecting variation in mortality rates in calves in GB since 2011. Alongside providing a first benchmark for calf mortality rates in GB, factors associated with mortality rates were further explored utilising multivariate adaptive regression spline models and suggest that environmental conditions such as mean monthly environmental temperature and month of birth play a significant role in calf mortality rates at a national level.

To identify the most important factors for optimal calf health and performance, the farm management practices were collected from 60 farms, resulting in a large number of potential housing and management variables affecting calf performance. In Chapter 3, an elastic net was used in combination with stability selection techniques, and these were utilised to identify which factors were most likely to improve calf performance on the majority of farms, and included management areas such as stocking demographics, milk/colostrum feeding, environmental hygiene and environmental temperature.

Colostrum samples were also collected from enrolled farms, and in Chapter 4, a first benchmark of bacterial levels within colostrum on GB dairy farms was provided. Bacterial levels in were significantly higher when colostrum was collected using equipment than taken directly from the cow's teat, suggesting interventions to reduce bacterial contamination should focus on the hygiene of collection and feeding equipment. An automated backwards stepwise mixed effects regression model was utilised in conjunction with stability selection to identify a small number of variables likely to have the largest effect on colostrum hygiene

on the largest number of farms and suggest that the cleaning of colostrum collection and feeding equipment after every use should be performed with hot water as opposed to cold water, and hypochlorite or peracetic acid as opposed to water or parlour wash.

It is important that associations identified during observational studies are not interpreted as causal, and the most important variables identified in Chapter 2, 3 and 4 were tested as a calf health plan intervention in a randomised controlled trial to elucidate causality, as described in Chapter 5. Health and performance outcomes were analysed for 60 dairy farms randomly allocated to receive the health plan as an intervention. Growth rates were higher for calves on farms receiving the plan for both male or beef and dairy heifer calves, and results from regression models suggest that male or beef calves had significant increased growth rates on farms receiving the plan than those that were not. Model predictions suggest that a farm with the highest number of interventions in place (15) compared to farms with the lowest number of interventions in place (4) would expect an improvement in mean growth rates from 0.65kg/d to 0.81kg/d for male or beef calves, from 0.73kg/d to 0.88kg/d for dairy heifers, a decrease in mortality rates from 10.9% to 2.8% in male or beef calves, and a decrease in diarrhoea rates from 42.1% to 15.1% in dairy heifers.

Neonatal calves are relatively susceptible to heat loss, and Chapters 2 and 3 suggested that reduced environmental temperatures are associated with increased calf mortality, and reduced growth rates. The aim of Chapter 6 was to evaluate the impact of calf jackets and supplementary heat sources on the growth rates of preweaned calves in a randomised controlled trial. Seventy-nine calves from a single British dairy farm were randomly allocated to receive heat lamps or calf jackets in a factorial study design. Regression model results suggest 1kW heat lamp usage significantly improved growth rates by around 90g/d, and no effect of jacket were identified. A significant, positive impact of increased pen temperature on calf ADG was also identified in this study and was reinforced when including prior information from Chapter 3 within a Bayesian framework.

The research presented in this thesis utilised a range of statistical learning techniques to identify factors associated with calf performance, which have been tested in a randomised controlled trial. To provide farmers and veterinarians with access to the calf health findings of the thesis in interactive form, the University of Nottingham Herd Health Toolkit (www.nottingham.ac.uk/herdhealthtoolkit) was created, including tools relating to the management of colostrum, prediction of mortality rates and ultimately a bespoke calf health plan based on user inputs. A number of statistical learning techniques within the field of stability selection were developed in parallel to this thesis, and the creation of the *stabiliser* R package to allow these techniques to be utilised by the wider research community.

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Chapter 1 Introduction

1.1. Background

The effective management of calves is one of the most important areas on dairy farms, and can have substantial impacts in terms of health, welfare, and productivity (Windeyer *et al.*, 2014). Incidence rates of neonatal calf diarrhoea and bovine respiratory disease (BRD) in UK dairy calves are relatively high (Johnson *et al.*, 2017), and have been highlighted in previous research to be a major driver of high antimicrobial usage in the dairy industry (Hyde et al. 2017). Concerns have been raised over the environmental impact of both dairy and beef production, and a high degree of heterogeneity in the environmental impact between producers has been identified (Poore and Nemecek, 2018), suggesting efficiency at farm level may play an important role in greenhouse gas emissions as well as productivity. Estimates of methane emissions for UK herds suggest replacement stock accounts for 21-26% of whole herd enteric methane emissions (Wall, Coffey and Pollott, 2012) and the efficient rearing of youngstock and subsequent reductions in the age at first calving have been suggested as a method of reducing whole-herd emissions (Knapp *et al.*, 2014). The rearing of replacement dairy heifers is also an expensive process, being estimated to cost around £1819±387/heifer in the UK (Boulton, Rushton and Wathes, 2017). Not only this, but the UK is the third largest producer of beef in Europe (DEFRA, 2018), of which over 50% comes from dairy-cross beef calves (AHDB, 2017), highlighting the importance of optimal calf health for both beef production and dairy heifer replacement.

"Herd health" is an approach to the veterinary care of production animals applied at a population level, rather than the individual animal level (Green, 2012). The focus of herd health is on improving animal health at a herd level, often using a data driven approach. Data driven tools can provide insights into issues related to calf management, with increased awareness being associated with decreased mortality (Santman-Berends *et al.*, 2021). Whilst youngstock health and performance is extremely important in both economic and efficiency terms, dairy farmers often rank youngstock performance as the least important issue when compared with adult dairy cow diseases such as lameness and fertility (Atkinson, 2015). Benchmarking calf performance within farmer groups has been shown to motivate farmer engagement in calf care (Atkinson, von Keyserlingk and Weary, 2017), and by providing farmers with better access to their data, more calf management problems might be addressed (Sumner, von Keyserlingk and Weary, 2018). The dairy mastitis control plan (DMCP) is an evidence-based method of reducing mastitis levels by around 20% (Green *et al.*, 2007), and was created using an epidemiological approach to identify key factors associated with

disease, which were later tested as an intervention in randomised controlled trial. The same methodology might be applied to the field of calf health and production as the research base develops.

There is an ever increasing of big data being collected within the veterinary field (VanderWaal *et al.*, 2017), and making the most of these data is crucial in the advancement of farm animal veterinary practice (Hudson, Kaler and Down, 2018). The majority of statistical techniques are intended for a low dimensional setting, where the number of observations is much greater than the number of available variables (James *et al.*, 2017), and conventional statistical approaches are often poorly suited to big data problems, particularly when the number of variables exceed the number of observations (Zou and Hastie, 2005). The number of potential variables available in this data rich environment is increasing, and although a wide number of statistical learning algorithms are being used to analyse factors associated with health and production outcomes on dairy farms (Slob, Catal and Kassahun, 2021), methods of robustly identifying variables that are truly associated with an outcome within a high dimensional setting are becoming increasingly important. It is not only crucial to apply robust statistical learning techniques for research data, but also to present the findings to veterinarians and farmers in a usable form to inform management changes. The following sections provide a review of the current literature around calf health and production, the pitfalls of conventional statistical techniques and solutions to these issues.

1.2. Calf health and productivity

1.2.1. Average daily gain

Growth rate during the preweaning phase is essential in the development and future performance of both dairy and dairy-cross beef calves on dairy farms. Average daily gain (ADG) has been shown to be a crucial factor in the efficient rearing of dairy heifers (Chester-Jones *et al.*, 2017) and has been associated with increased milk yield during first lactation (Soberon *et al.*, 2012; Gelsinger, Heinrichs and Jones, 2016).

ADG in Great Britain (GB) in recent studies have been reported as a mean of 0.73kg/d up to 138d (Archer, 2021), relatively similar to previous GB study findings of 0.77kg/d up to 6months of age (Brickell, McGowan and Wathes, 2009) and 0.71kg/d over 730d (Bazeley *et al.*, 2016). Relatively high levels of variability between both farms and calves has been noted (Brickell, McGowan and Wathes, 2009), although the effect of veterinary practice has been reported to explain a relatively low proportion of variation in calf weight (Archer, 2021), potentially suggesting that veterinary input has had a limited effect in improving ADG.

Increases in ADG during the preweaning period may result in significant improvements in first lactation milk yield, and in a large UK study the estimated improvement in mean first lactation milk yield when improving mean growth rates from the poorest performing 2.5% to the best performing 2.5% of farms would be 682+/-280kg (Archer, 2021) based on estimates from previous studies (Soberon *et al.*, 2012). However, very high growth rates have been associated with a negative effect on mammary gland development in some studies (Meyer *et al.*, 2006), although more recent studies indicate that this negative correlation may not be the same throughout the prepubertal period and a greater rate of gain in the preweaning period is likely to be beneficial to mammary gland development (Soberon et al., 2012). Subsequent literature suggest that calves can safely consume around 20% of body weight, with greater milk consumption supporting a greater ADG, reduced incidence of disease and potentially improved welfare (Khan et al., 2011), and the optimal prepubertal ADG has been reported as 0.8kg/d (Zanton and Heinrichs, 2005); a realistic goal for most producers.

Nutrition is likely to play an important role in ADG, both in terms of quantity and quality of feed provided. Higher planes of nutrition in calves have received much attention, and caloric requirements are of crucial importance to calf productivity, particularly in cold environments (National Research Council, 2001). Milk allowance has been shown to not only have an effect on weight gain that can persist beyond weaning, but also has an effect on behaviour, with calves being fed less milk visiting the feeder more often (Rosenberger *et al.*, 2017). Some studies however report no effect of milk replacer feeding rate and age of weaning on bodyweight and hip width at 112d, also noting that gradual weaning had no effect on postweaning digestion (Dennis *et al.*, 2018); particularly important given studies reporting that starter feed and dry matter intake was lower in preweaned calves when fed a high plane of nutrition preweaning (Mirzaei *et al.*, 2018). Some studies have also reported also reported no difference in body weight or hip width from 0-4 months when fed a high quantity of milk replacer (>0.66kg DM) during the preweaning period (Hill *et al.*, 2016). Later studies have suggested that if feeding a higher plane of nutrition preweaning, it may be advantageous to wean at a later date (75 vs 60d) to improve wither and hip height (Mirzaei *et al.*, 2018). Further studies exploring feed source include the use of free access acidified milk, which has been compared with 6L milk replacer resulting in initially an improved ADG and lower disease rates (Todd *et al.*, 2017). This has reinforced previous findings that have shown reduced immune response related to inadequate nutrition (Pollock *et al.*, 1993) suggesting there is also likely to be an association between nutrition and both morbidity and mortality outcomes. Whilst growth rates are likely to be important, it is also worth considering the most efficient methods of growing calves from a feed efficiency and economic standpoint. As feed efficiency decreases over time, but feed costs also increase as the proportion of

dietary forage intake increases, and consequently it is likely to be economically advantageous to maximise growth rates either preweaning, or immediately after weaning (Bach, Ahedo and Kertz, 2021).

In addition to the effect of milk feeding volume on ADG, the type of feed being fed during the preweaning period is also of interest. Despite the relatively common practice of feeding artificial milk replacer powder, the feeding of whole milk has been associated with increased growth during the preweaning period compared with milk replacer powder, and increased first lactation milk yield, potentially related to milk supply, paracrine or endocrine effects of fat tissues on mammary parenchyma, or a combination of both factors (Moallem *et al.*, 2010). Whilst concerns exist around the potential for transmission of diseases such as *Mycobacterium avium subspecies paratuberculosis* to calves via whole milk, recent literature reviewing cow-calf separation suggest there is minimal evidence to support these concerns (Beaver *et al.*, 2019). The source of whole milk however is likely to be important, and the feeding of antibiotic waste milk has been shown to increase the prevalence of antimicrobial resistance to several antibiotics including the critically important 3rd generation cephalosporins (Maynou *et al.*, 2017), and increasing levels of resistance in nasal *Pasteurella multocida* (Maynou, Bach and Terré, 2017). Alongside milk intake, there are concerns around water provision, and a previous study of 102 UK dairy farms has suggested that 22% of farms might not provide water to calves within seven days of birth (Boulton, Rushton and Wathes, 2015). Systematic reviews into the use of probiotics to improve health and productivity of calves suggests calves fed probiotics had higher ADG during the preweaning period (Alawneh *et al.*, 2020), although the evidence for improved health/risk of disease is relatively weak. A meta-analysis of the effects of preweaned calf nutrition on first lactation performance ultimately determined that other aspects of management were far more important than preweaning intake and ADG but did find a synergy between preweaning liquid/starter dry matter intake for improving production, and that ADG >0.5kg can enhance first lactation performance when combined with proper management postweaning (Gelsinger, Heinrichs and Jones, 2016).

Studies exploring the effects of housing and management on ADG report a variety of factors associated with increased growth rates, such as an increased space in wooden hutches being associated with better ADG, bodyweights pre- and post-weaning, and less faeces present on the calf's body (Calvo-Lorenzo *et al.*, 2016). Not only is the environment important in terms of the housing facilities and hygiene, but social dynamics may also impact ADG. A review into group housing found that social housing improved solid feed and ADG before and after weaning, and that whilst concerns exist around group housing and increased disease transmission, these risks can be mitigated by good management (Costa, von Keyserlingk and

Weary, 2016). Similarly, a separate review reported that environmental enrichment can improve lifetime reproductive success alongside behavioural benefits (Mandel *et al.*, 2016). Housing group changes have been shown to be negatively associated with growth performance (Tautenhahn, Merle and Müller, 2020), likely relating to the negative effects of social group changes through the preweaning period. This is further compounded by studies of pair vs individual housing, reporting a benefit of increased starter intake, and reduced neophobia, in pair housed calves, although there was no significant difference in bodyweight (Whalin, Weary and von Keyserlingk, 2018). Although concerns exist around the logistical challenges of adapting calf rearing systems from single housing to pair housing, studies have demonstrated effective methods of creating pair housing systems from individual calf hutches (Wormsbecher *et al.*, 2017). During early life, a large proportion of the variation in growth rates explained has been shown to be at calf level rather than farm level (Archer, 2021), and although the variation is explained more by farm past 66d of age, it is likely that individual, rather than farm level factors play a large role during the preweaning period. A particular factor associated with reductions in growth rates is the incidence of disease such diarrhoea within the first 3 months of life (Windeyer *et al.*, 2014).

Achieving optimum growth rates in calves is important in achieving an optimum age at first calving and is likely to affect future production in dairy heifers. Improving growth rates on farms is likely to be multifactorial, and the provision of high-quality nutrition at a relatively high rate, in combination with a clean, dry, and warm environment is likely to be important. Whilst there are previous studies exploring factors associated with growth rates in preweaned calves, there are a large number of farm management factors that have not been explored previously, and many factors have not been evaluated in a randomised controlled trial.

1.2.2. Mortality

Mortality in calves is often defined as either perinatal (0-1d of age), or neonatal (1day of age to weaning) mortality (Compton *et al.*, 2017). Mortality in dairy cattle of all ages has a significant impact both in financial and welfare terms, and the incidence risk of perinatal, but not neonatal mortality had increased significantly over time for dairy calves (Compton *et al.*, 2017). There are a number of challenges in defining mortality rates (Santman-Berends, Schukken and van Schaik, 2019), not least the issue recording perinatal deaths (often termed as “stillbirths”), and whilst proposals have been suggested for the recording of these cases (Lombard *et al.*, 2019), exact figures are often challenging to obtain.

Levels of neonatal calf mortality in GB have been recently estimated at 4.5% between 1d-2mo of age, for dairy calves from 11 herds (Johnson *et al.*, 2017) in 2011-2012. National

level data from 2007 suggested an on-farm 0-6 month mortality rate of 2.47% for beef and 7.42% for dairy calves (Gates, 2013). These data, however, are relatively historic, and despite the obligation for keepers of bovine animals to register all births, deaths and movements through the national Cattle Tracing System, the data are not used to routinely report national incidence rates of calf mortality and the current levels of calf mortality in GB at a national level are currently unknown.

Alongside knowledge gaps in the overall calf mortality rates at a national level, there is also no information as to the mortality rates of particular demographic groups of calves. Certain groups of calves might experience higher rates of mortality, and whilst the increased use of sexed semen has resulted in an increasing number of surplus female calves (Bolton and von Keyserlingk, 2021), male dairy calves often have an increased mortality rate compared with their female counterparts (Cuttance and Laven, 2019). Studies have suggested that a participatory approach is needed to develop sustainable solutions to the issue of surplus calves (Bolton and von Keyserlingk, 2021), and studies have suggested that farmers often either not aware or felt powerless to find a solution to mortality problems (Santman-Berends *et al.*, 2014).

A number of factors have been reported to be associated with mortality in preweaned calves, and data driven tools to reduce mortality levels have been utilised in the Netherlands (Santman-Berends *et al.*, 2021) resulting in a relative decrease in mortality rates of 18% in preweaned calves. Factors associated with calf mortality often relate to hygiene and immunity, and the pasteurisation of colostrum and milk has been shown to effectively improve health status and decrease mortality, even when calves are already receiving appropriate quantities colostrum (Armengol and Fraile, 2016). One Finnish study found an association between calf mortality rate <7d with larger herd size and not separating sick calves, and a 7-180d mortality rate associated with a shorter whole milk feeding period and lower production, also finding that mortality risk for calves was lower on farms where a veterinarian performed disbudding rather than the farmer (Seppä-Lassila *et al.*, 2016). A Norwegian study found an association between the absence of water access and higher calf mortality rates (Johnsen *et al.*, 2021). Nutrition also plays a part, and the feeding of low fat during the preweaning period also increased the risk of mortality (Urie *et al.*, 2018), and greater body weight on arrival to veal facilities was associated with a reduction in early mortality (Renaud *et al.*, 2018). Colostrum management is likely a major factor involved in early calf mortality, with a meta-analysis examining failure of passive transfer found an adjusted risk for mortality of 2.12 (95% CI 1.42-3.13) for calves with failure of passive transfer (Raboisson, Trillat and Cahuzac, 2016). Tube and pail feeding of colostrum, the use of shaving/straw bedding, and the absence of active/routine calf health involvement from a

veterinarian are all associated with higher mortality, and the regular checking of calving pens every 3hrs or more frequently was associated with reduced odds of being a high mortality farm (Renaud *et al*., 2018).

Factors associated with calf mortality in previous literature often centre on hygiene, both of the environment and of colostrum. Despite the legal requirement to record all births, deaths and movements through the national Cattle Tracing System, current mortality rates in GB for calves is currently unknown, and this national data source represents great potential for epidemiological studies to identify factors associated with calf mortality at a national level.

1.2.3. Bovine respiratory disease

Bovine respiratory disease (BRD) is reported to be the most important disease in calves greater than 30 days of age (McGuirk, 2008), and in UK herds, respiratory disease has been reported to affect 46% of calves and be accountable for around one-third of mortality (Johnson *et al.*, 2017). BRD is associated with abnormal posture, isolation from the group and lethargy (Cramer, Ollivett and Stanton, 2016), although the effective identification of BRD can be challenging in practice. Commonly reported pathogens include bovine respiratory syncytial virus, infectious bovine rhinotracheitis virus and *Manhemia haemolytica*, and studies have also reported other pathogens such as *Moraxella catarrhalis*, which is known to cause pneumonia in humans (Gaeta *et al.*, 2017). Whilst there are many pathogens that might cause BRD, there is likely to be a significant environmental component underlying most cases. Estimations of disease data from farmer recorded data has been reported to have several caveats, with studies demonstrating only moderate agreement between farmer and veterinary diagnosis of BRD (Sivula *et al.*, 1996). Studies have also shown that whilst veterinary diagnosis of pneumonia was associated with reduced liveweight gain, there was no significant difference when diagnosed by a farmer (Virtala *et al.*, 1996). Recent publications have also suggested that scoring systems applied on a weekly basis may be insufficient to detect acute onset problems (Mahendran *et al.*, 2017). The early treatment of pneumonia is likely to increase the chance of cure, however, one study reported that more than half of BRD cases did not receive antibiotic treatment upon detection (Svensson *et al.*, 2003). All of which calls into question the validity of disease information supplied by producers (Johnson *et al.*, 2017), and accurate scoring systems depend on regularly scoring at least twice weekly (Mahendran *et al.*, 2017). Whilst farmer recorded disease has its limitations, the advantages of benchmarking producers alongside each other is clear, and has been shown to have benefits in increasing ADG (Atkinson, von Keyserlingk and Weary, 2017).

Thoracic ultrasound is a potential tool that has been suggested as providing improved disease detection rates. Lung consolidation as assessed by ultrasound in the first 8 weeks of life has been associated with a reduction in first lactation 305d yield of 525kg, however was not found to affect age at first calving or survival (Dunn *et al.*, 2018). Thoracic ultrasound diagnosed lung consolidation in other studies demonstrated that whilst there was no significant difference in mortality between 60-350d or milk yield, there was a significantly higher hazard of removal from the herd between 350d-first calving, and a lower hazard of pregnancy (Teixeira, McArt and Bicalho, 2017). Similarly, studies have found that extensive consolidation is associated with a higher risk of dying and culling than no abnormalities (Adams and Buczinski, 2016).

Studies have demonstrated that an increased incidence of BRD is associated with the sharing of airspace with older cattle and a greater depth of wet bedding pack (Medrano-Galarza *et al.*, 2018). Studies in Australian feedlot cattle reported an increased risk of BRD when water troughs were present between adjoining pens, and the addition of animals over multiple days, however, finding no significant effect of IBR vaccination, and a reduced risk of BRD if the cattle were previously yard weaned compared with pasture, grain fed, or vaccinated for either bovine viral diarrhoea virus (BVD) or *Manheimia* (Hay *et al.*, 2016). When examined at population level, the most important intervention risk factors were shared pen water, breed, history of previous mixing with cattle from other herds, and the presence of BVD within the animals' cohort, with an estimated 82% of BRD incidence being attributable to management related risk factors (Hay *et al.*, 2017). A retrospective study examining risk factors for a primary necropsy diagnosis of BRD, suggested that male cattle >31d were most at risk, and that risk increased with herd size, and decreased with a longer time since the last animal movement onto the holding (Murray *et al.*, 2016). There may also be a nutritional component to BRD, with some research indicating a reduction in BRD in calves fed whole milk vs milk replacer, and increased solids in milk (Medrano-Galarza *et al.*, 2018). A previous review suggested that the control of BRD should focus on achieving adequate passive transfer as well as improving environmental conditions and nutrition (for example improved ventilation, increased frequency of bedding and adequate energy intake) (Gorden and Plummer, 2010). A large study of 10,179 calves in the upper Midwest USA (Jorgensen *et al.*, 2017) suggested that bacterial counts in milk were associated with attitude, ear, and fever scores. Higher peak milk allowance was increased with lower hide dirtiness, and less space per calf was associated with higher ear and eye scores. Larger group sizes were associated with higher nasal scores, and rectangular pens were associated with decreased eye sores.

An extensive range of vaccines are available for the common causes of respiratory disease, although are unlikely to completely prevent disease in the presence of poor environmental conditions (Sherwin and Down, 2018). Large, randomised control trials have demonstrated no effect of vaccination on the risk of BRD or mortality in calves monitored to 3 months of age (Windeyer *et al.*, 2012). Recent research into intranasal vaccines have reported mixed results, and whilst an effect has been demonstrated on antibody levels (Hill *et al.*, 2012), studies have demonstrated no effect on the incidence of disease in calves (Ollivett *et al.*, 2018). Alternate methods to reduce the risk of early calf BRD could be to vaccinate dams, and whilst this has been shown to increase protection to specific respiratory pathogens (Makoschey *et al.*, 2012), it is limited by the requirement for successful immune transfer via colostrum (Windeyer, Timsit and Barkema, 2017) which is inadequate on many farms.

There has been a trend recently towards group housing of preweaned calves for a number of reasons, including the improved ability to develop natural behaviours, increased automatic feeding, and improved working conditions for labourers (Windeyer, Timsit and Barkema, 2017). Whilst concerns around increased spread of contagious disease in the group housed environment exist, research would suggest that small group housing has no significant disadvantage in morbidity compared with individual housing (Johnson, 2011). The ventilation of calf barns has been suggested as being highly important in respiratory disease control (Lago *et al.*, 2006), however there are several challenges with the objective monitoring of ventilation in livestock buildings. Monitoring of noxious gases has previously been used to monitor ventilation in livestock housing (Feddes, Leonard and McQuitty, 1984; Gerber *et al.*, 1991), however preliminary investigations in naturally ventilated calf housing showed minimal variation in concentrations of carbon dioxide and ammonia between barns (Lago *et al.*, 2006). The optimal ventilation system is far from proven, with some studies reporting increased odds of developing disease in positive/cross ventilated buildings compared with naturally ventilated sheds with studies finding that naturally ventilating calf buildings are often microclimates of poor air hygiene (Lago *et al*., 2006). This is further compounded by research finding that the absence of positive pressure ventilation tubes was associated with more fevers (Jorgensen *et al.*, 2017).

Environmental temperature has been widely reported to be essential to calf health, and studies performed in extremely hot temperatures report a positive correlation between the maximum daily temperature and BRD (Louie *et al.*, 2018). Other studies have reported an inverse relationship between temperature humidity index and morbidity (Urie *et al.*, 2018), likely highlighting that the relationship between temperature/humidity and disease is far from linear. Research has demonstrated associations between meteorological conditions and the hutch environment (Louie *et al.*, 2018), suggesting data from local weather stations

might be used to predict potentially stressful conditions for the calves, whilst noting that on farm weather stations are likely to be superior in predicting BRD risks. However, one study however has found significant differences between on-farm environmental conditions from data loggers placed in adult cow pens and local weather stations (Shock *et al.*, 2016). Recent studies have found a higher mean temperature in the first month of birth was associated with lower odds of BRD in the first 9 weeks of life (Johnson, Chancellor and Wathes, 2021).

Pneumonia in preweaned calves continues to be common in GB, with serious ramifications in terms of both welfare and future productivity. There are many factors reported to be associated with pneumonia, however accurate disease detection remains a significant limitation to research in this area.

1.2.4. Diarrhoea

Diarrhoea has been reported to be the most important disease in calves less than 30 days of age (McGuirk, 2008), consistent with a recent study in UK herds which reported 48% of calves being diagnosed with diarrhoea, which was accountable for around one-third of mortality (Johnson *et al.*, 2017). Calves that do succumb to diarrhoea are likely to perform poorly, and have the potential to die, with low serum total proteins and the lack of a suckle reflex being major risk factors associated with case fatality in diarrhoeic calves (Boccardo *et al.*, 2017). Alongside the impacts of diarrhoea on animal health and welfare, there is also a zoonotic potential for farm or veterinary workers that bears consideration (Heller and Chigerwe, 2018).

The detection of diarrhoea by farm staff is essential to ensure prompt treatment, and faecal score has been reported to be an effective method of detecting diarrhoea, with general calf cleanliness proving less effective (Graham *et al.*, 2018). The use of algorithms for selection of treatment of diarrhoeic calves has been shown to reduce antimicrobial usage (AMU) without negative impacts on calf health (Gomez *et al.*, 2017). Similarly to pneumonia, there are a range of pathogens that can cause bovine neonatal diarrhoea such as *E. coli* and *cryptosporidium*, however other pathogens such as bovine norovirus are also frequently detected in calf diarrhoea and represent a potential missing component of calf diarrhoea diagnostics (Di Felice *et al.*, 2016). The use of calf-side diagnostics can help identify predominant pathogens in cases of calf diarrhoea, though mixed infections are commonly present and diarrhoea pathogens ubiquitous.

Diarrhoea is an important disease in calf management, and similarly to pneumonia, likely has a significant environmental component, with a large Ontario study of 1,488 calves reporting lower diarrhoea prevalence associated with frequent provision of fresh bedding

every 2-3d. Higher diarrhoea was associated with the sharing of airspace with older cattle and high bacterial counts in milk (>100,000 cfu/ml) during the summer. (Medrano-Galarza *et al.*, 2018). The use of maternity pens for anything other than calving has been associated with an increase in both diarrhoea and BRD in calves (Medrano-Galarza *et al.*, 2018).

A previous study evaluated protocols to reduce neonatal calf diarrhoea based largely on increased vaccination against *E. coli*, *rotavirus*, and *coronavirus*, and attempting to ameliorate the effects of C*ryptosporidium* through the application of halofuginone showing a reduction in the percentage of calves suffering from neonatal diarrhoea (39.7% versus 14.3%) across 24 dairy farms (Meganck *et al.*, 2015).

Reviews into effective methods of diarrhoea control maximising calf immunity through effective passive transfer of immunity from colostrum and reducing the pathogen challenge by improving environmental hygiene are likely to be the most effective method of prevention (Lorenz *et al.*, 2011). There are a wide range of potential factors associated with diarrhoea in preweaned calves, and these have not been examined in a holistic manner, nor have they been tested as a randomised controlled trial to elucidate causality.

1.2.5. Colostrum

Colostrum management is of vital importance to calf health and productivity, and the failure of passive transfer (FPT as indicated by serum IgG <10g/L) results in higher morbidity and mortality within 30days of age (Lora *et al.*, 2018), also being associated with a cost per calf of a case of FPT of 60 euro and 80 euro for dairy and beef respectively (Raboisson, Trillat and Cahuzac, 2016). Recent studies into failure of passive transfer rates in the UK reported 21% of 492 calves from 11 farms (Johnson *et al.*, 2017) to 26% of 444 calves from seven farms (MacFarlane *et al.*, 2015) failed to receive adequate immunoglobulin transfer. The timing of colostrum feeding is crucial, and a study comparing 45min, 6hr and 12hr feeding times post calving, found delayed feeding of colostrum not only reduced efficiency of IgG absorption, but also decreased the prevalence of *E. coli* in ileum mucosa (Fischer *et al.*, 2018). The addition of supplementary colostrum during early life has also been hypothesised to be beneficial but has been demonstrated to have no effect on IgG or ADG after being fed for 14d, however, it did result in less abnormal faeces or respiration, as well as calves being less likely to receive antibiotics (Chamorro, Cernicchiaro and Haines, 2017).

Colostrum quality has a significant bearing on passive transfer of immunity, and a meta-analysis has demonstrated that colostrum brix values of ≥22% should be considered “good quality”, and <18% should be discarded, and colostrum should be supplemented when between the two(Buczinski and Vandeweerd, 2016).

The monitoring and benchmarking of FPT levels on farms often relies on calf blood samples to measure transfer of immunity. The cut-off points of serum total proteins as a proxy for passive immunity transfer vary between studies, with higher levels (5.7g/dl) being shown to be predictive of BRD, and >5.2g/dl for mortality within 5 weeks (Windeyer *et al.*, 2014). Infrared spectroscopy has been shown to be slightly more sensitive and specific than digital and optical spectroscopy at determining colostrum quality, however, being lab-based is a significant disadvantage in clinical practice (Elsohaby *et al.*, 2017). The quantity of colostrum fed is also important, with studies reporting 8.5% of calf bodyweight being optimal for the effective passive transfer of immunity (Conneely *et al.*, 2014).

Hygienic management of colostrum is vital, and high levels of bacteria have been shown to reduce IgG absorption efficiency and consequently serum total protein levels. Heat treatment improved plasma IgG levels at 48hr, and whilst heat treated colostrum with lower bacterial levels tended to result in fewer days of scour, there was no significant difference in respiratory scores (Gelsinger, Jones and Heinrichs, 2015). Previous authors have suggested the total bacterial count should not exceed 100,000 cfu/ml, with coliform counts being below 10,000 cfu/ml (McGuirk and Collins, 2004). Studies investigating colostrum equipment cleanliness have reported the use of some commercial luminometry swabs are appropriate for assessing coliform counts in colostrum feeding equipment but note that visual assessment and some swabs is not appropriate. (Renaud *et al.*, 2017). High pressure treatment of colostrum at 400MPa for 15minutes has been shown to reduce bacterial counts (excluding MAP), whilst maintaining IgG concentrations at acceptable levels (Foster *et al.*, 2016), although this is currently uncommon in the UK. Other environmental/management factors have been reported to be important for colostrum management, with studies reporting a positive effect on passive transfer rates through increased time spent in the calving pen (Johnson *et al.*, 2017) in contrast with previous studies reporting an increased time spent within the calving pen to be consistently detrimental to passive transfer, along with a strong seasonal effect (McAloon *et al.*, 2016). Calves in seasonal calving pasture-based herds with failure of passive transfer had increased odds of a farmer recorded health event (predominantly diarrhoea) preweaning (OR 1.68) and of mortality by 6mo (OR 2.19) and 12mo (OR 2.21) and had lower bodyweights at weaning and 12mo (Cuttance *et al.*, 2018), although the authors comment that this may vary compared with housed herds.

One potential option for ensuring supply of colostrum to calves is artificial colostrum replacer, and studies have demonstrated that whilst calves fed colostrum replacer were slightly lighter at weaning, and had numerically more cases of diarrhoea, there was no significant difference in mortality, making colostrum replacer a valid alternative to maternal colostrum (Lago *et al.*, 2018). Whilst colostrum replacer had lower levels of contamination

as measured by coliform count, there was no significance in IgG absorption efficiency (Lago *et al.*, 2018).

Storage of colostrum is clearly important, and even more so considering the potential of a single freeze thaw cycle to have a 1 log reduction in *M. bovis* (Gille *et al.*, 2018). Storing colostrum at 4°C resulted in more bacteria being present than fresh colostrum, however no differences were apparent in IgG and ADG, leading the authors to conclude that storage ≤4°C is acceptable if colostrum cannot be fed immediately (Cummins *et al.*, 2017).

Transmission of *Mycobacterium avium subspecies paratuberculosis* (MAP) to calves is often of concern, with reviews suggesting that calves whilst calves are susceptible up to 1yr old, lesions are far worse when inoculated at a younger age (Mortier, Barkema and De Buck, 2015). The importance of colostrum management in the control of MAP has been called into question by studies demonstrating no effect of colostrum pasteurisation on not only future yield or leaving the herd, but also no difference in MAP by both ELISA and faecal culture after 3 years (Godden *et al.*, 2015). In addition to this, research has demonstrated evidence of MAP presence within a high proportion (31%) of milk replacer powders, meaning calves may be exposed to MAP regardless of colostrum fed (Grant *et al.*, 2017).

Colostrum management remains a crucial component of neonatal calf management and has significant impacts on the health and future productivity of calves. Current levels of bacterial contamination in GB are unknown, and factors associated with high levels of contamination have not been fully explored.

1.3. Statistical learning

Establishing the key factors that influence disease variability is a fundamental part of the epidemiological approach (Dohoo, Martin and Stryhn, 2009), and the analysis of many management variables to identify factors associated with a calf health or production outcome has historically employed conventional statistical techniques such as regression models. Inferential epidemiological research often involves the identification of causal variables from a relatively high dimensional space (Lima, Hyde and Green, 2021), where conventional regression models are likely to perform poorly (Greenland, 2007; Kuhn and Johnson, 2013). Health and productivity outcomes in calves are likely to be multifactorial (Windeyer *et al.*, 2014), with many variables likely to be available at both farm and calf level (Archer, 2021). Given the high number of potential management variables a calf or farm might experience, it is vital that statistical modelling approaches are able to sufficiently deal with large numbers of explanator variables, even in datasets with relatively low numbers of observations.

Statistical learning (or machine learning) algorithms have a long history in a variety of applications from the filtering of spam emails (Guzella and Caminhas, 2009) to the suggestion of movies a Netflix user might next enjoy (Koren, 2009; Töscher, Jahrer and Bell, 2009), and have been increasingly used in the field of dairy research (Slob, Catal and Kassahun, 2021). Statistical learning algorithms can be thought of as approaching problems like a veterinary or medical practitioner progressing through training might; learning rules from data and applying them to new patients and situations (Obermeyer and Emanuel, 2016). The application of statistical learning in human medicine is relatively broad, ranging from the accurate classification of skin cancer (Esteva *et al.*, 2017), and retinal disease (Fauw and Ledsam, 2018) through image recognition, to the diagnosis of diabetes (Barakat et al., 2010) and cardiac arrythmia (Özçift, 2011). Although there is a relatively large body of research in the use of machine learning in the field of medicine, examples of translation into an effect on clinical management are relatively rare (Clifton *et al.*, 2015). It has been recognised that unless there is a collaborative approach between data scientists and clinicians to integrate the practice into real world settings, there will be minimal impact on patient care (Lynch and Liston, 2018).

Statistical learning in cattle medicine has been utilised at a herd level to predict bovine viral diarrhoea virus exposure (Machado, Mendoza and Corbellini, 2015), the distribution of exposure of herds to liver fluke (Ducheyne *et al.*, 2015), and in the replication of complex specialist clinical veterinary decisions in an automated method of determining mastitis origin (Hyde *et al.*, 2020). It has also been applied at an individual level in the prediction of fertility outcomes (Fenlon *et al.*, 2016), high somatic cell counts (Ebrahimie *et al.*, 2018), and the prediction of calving (Fenlon *et al.*, 2017). Examples of these techniques within the field of calf health however are rare.

1.4. Conventional approaches

1.4.1. Regression modelling

Research aiming to identify factors associated with an outcome often involve datasets including an outcome of interest, and a number of potentially related variables. Two major questions exist; which variables are likely to be associated with the outcome, and what effect might they have. A typical approach to identify variables likely to be associated with an outcome in research is the use of linear or logistic regression modelling. Conventional modelling approaches often include linear modelling using the following structure

$$Y = \mu + \sum_{i=1}^{p} \beta_i x_i + \epsilon,$$

where *Y* is the outcome of interest, μ the intercept, x_i the *i*th of *p* covariates with an estimated coefficient β_i and ϵ the residual model error. A common method of fitting a linear model is to do so using the method of least squares such as

$$RSS(\beta) = \sum_{i=1}^{N} (y_i - f(x_i))^2,$$

where the difference between each value of *y* and $f(x)$ (or $\hat{y}$) is calculated as the residual, and the sum of all residual values squared would total the residual sum of squares (RSS), and values for coefficient β would be chosen to minimise the RSS (James *et al.*, 2017), providing an estimation of the effect a given variable has on the outcome of interest.

Not all variables are likely to be associated with a given outcome, and stepwise selection procedures are often utilised to determine which variables should be retained or excluded from a given model. Forward and backwards stepwise selection are often used (Zhang, 2016), by adding or removing variables in a stepwise manner until the "best" model is found by a given criterion, or by utilising hybrid approaches that include both backwards and forwards approaches simultaneously (Szulc, 2019). This iterative stepwise procedure can use a variety of criteria to identify the "best" model, often by minimising the root mean squared error (RMSE) or mean absolute error (MAE) of residual values. A common approach to comparing models is to identify a final set of variables by minimising the Akaike information criterion (AIC) using

$$\frac{1}{\mathrm{n}\hat{\sigma}^2}(RSS + 2d\hat{\sigma}^2),$$

where $\hat{\sigma}^2$ references an estimate of the variance of error ϵ associated with each response measurement for *d* predictors, and RSS the residual sum of squares. Another approach is the Bayesian information criterion (BIC) which tends to more heavily penalise complex models (James *et al.*, 2017), using

$$\frac{1}{\mathrm{n}\hat{\sigma}^2}(RSS + \log(n)d\hat{\sigma}^2).$$

A conventional approach to identifying management variables associated with a health or production outcome might be to perform iterative stepwise selection on a linear or logistic regression model, removing non-significant variables until the optimum set of variables were identified to provide the "best" model by a given metric such as AIC or BIC.

1.4.2. Conventional modelling pitfalls

There are a number of potential pitfalls to the use of conventional linear or logistic regression models when attempting to identify variables associated with an outcome, particularly from a higher dimensional space where there may be a large number of variables to consider, often with a relatively low number of observations. Firstly, conventional modelling approaches can erroneously remove potentially causal variables through stepwise selection. These analyses of course are not credible, as they assume that removed coefficients are "zero" and that they have no effect on the outcome at all. This in itself is a potential risk for net confounding, as the sum of these missed effects may be considerable. One solution is the use of "shrinkage" methods that, rather than removing variables altogether, reduce coefficients to near zero (in the case of ridge regression) or zero (in the case of lasso regression) (Zou and Hastie, 2005) as discussed in subsequent sections. Confidence intervals using stepwise approaches are known to be overly narrow (Greenland, 2007) and confidence intervals are likely to be wider for these shrinkage-based methods than conventional methods. This reflects the incorporation of further sources of uncertainty such as unmeasured confounders and lead to greater caution about inference than conventional regression methods, which make an unwarranted assumption that certain variables have "zero" effect on an outcome, translating into an overly narrow confidence interval. In addition, the somewhat arbitrary threshold of $p<0.05$ commonly used for "statistical significance" means many variables might be erroneously disregarded as being "non-significant", which has been recognised as a common, major flaw in this approach (Amrhein, Greenland and McShane, 2019).

Greenland (2007) discusses a specific example involving a breast cancer dataset, illustrating that shrinkage methods can far outperform conventional model approaches, and demonstrating the risks of stepwise deletion based on arbitrary thresholds in both the erroneous discarding of variables, the falsely inflated coefficients and overly narrow confidence intervals for coefficients. The consequences of these errors in determining variables associated with breast cancer are potentially severe and is also relevant to studies within the field of farm animal health, where the goal is often to identify causal management variables associated with a given health or production outcome. Erroneously discarding variables, falsely inflating coefficients and having overly narrow confidence intervals has major ramifications both in terms of the practical outcomes from a trial, and future research.

1.5. Beyond linearity

Whilst linear regression models are often utilised for continuous outcomes, relationships between variables and outcomes in epidemiological studies are often non-linear. Methods such as Multivariate adaptive regression splines (MARS) present a potential alternative for flexible regression modelling (Friedman, 1991) that move beyond linearity. MARS models can be described as

$$Y = \sum_{i=1}^{p} c_i B_i(x),$$

where c_i is the coefficient and B_i the basis function for the *i*th of *p* covariates, where the basis function can be one of either an intercept, hinge function or product of multiple hinge functions. Hinge functions take the form max(0, $x - c$) or max(0, $c - x$), where *c* is a constant, with the MARS model automatically determining the constant used for the hinge location, in effect creating several linear regression fits at a variety of points (Kuhn and Johnson, 2013).

Whilst MARS models can combine recursive partitioning methods (splitting into subpopulations using based on dichotomous variables) and spline fitting (being able to account for non-linear relationships between variables through the use of "hinges"), they still suffer from the so-called "curse of dimensionality", where an increase in explanatory variables can make the identification of significant variables challenging in the absence of a large number of observations (Bellman, 1961).

Other non-linear approaches such as Bayesian approaches allows the incorporation of both current (likelihood) and prior data to form a final estimate (posterior) of an association between a variable and a given outcome (Spiegelhalter, Abrams and Myles, 2004). The accuracy of an estimate of associations between a given variable and an outcome is likely to be improved when incorporating both new data and previous data where it is available in a Bayesian framework. A Bayesian model can be described as

$$p(\theta|y,x) \propto p(y|\theta,x)p(\theta|x),$$

where the posterior $p(\theta|y,x)$ is the join probability distribution of prior $p(\theta|x)$ and likelihood $p(y|\theta,x)$ for the outcome (θ) given the observed predictors (x) and data (y) (Muth, Oravecz and Gabry, 2018). Bayesian approaches have several potential advantages, in particular the ease with which data from previous experiments might be incorporated with more recent findings.

1.6. The high dimensionality problem

Datasets containing large numbers of explanatory variables are more likely to contain potentially spurious correlations, which can confound conventional modelling approaches, resulting in inflated coefficients and over fitting, particularly when the number of predictors (p) exceeds that of the observations (n) (Kuhn and Johnson, 2013; Hastie, Tibshirani and Wainwright, 2015). Overfitting where p>>n is clearly of great importance, particularly when using more traditional approaches such as conventional regression models which would be extremely likely to overfit. Stepwise regression models are likely to perform poorly in relatively small samples, firstly in the selection of truly causal variables, but also in the estimation of regression coefficients; particularly important when considering conditional selection bias and excessive leverage from extreme values (Dormann *et al.*, 2013).

Variable selection to reduce the number of variables is highly desirable, resulting in a parsimonious model without compromising predictive performance. Some model types such as support vector and neural network algorithms can be sensitive to irrelevant predictors, where others such as linear and logistic regression models can be sensitive to multicollinearity (Kuhn and Johnson, 2019), both of which can be ameliorated to at least some degree by variable selection. In addition to models with built-in feature selection, methods can largely be categorised as either *wrapper* methods or *filter* (John, Kohavi and Pfleger, 1994). *Wrapper* methods add or remove predictor variables to find the combination of variables that optimise model performance, whereas *filter* methods determine variable relevance through a pre-selection process prior to model building (Kuhn and Johnson, 2013). Ad-hoc *wrapper* methods of feature selection such as the removal of coefficients with little impact on the model and refitting is known as recursive feature elimination (Guyon *et al.*, 2002), but do not always perform well (Hastie, Tibshirani and Friedman, 2009) and can be more computationally expensive than filter methods (Kuhn and Johnson, 2013).

An issue with model building is the importance of achieving both a low degree of error between the average predicted value and the true value (bias) and a low degree of overfitting (variance). As model complexity increases the variance often increases despite bias decreasing; the so-called variance: bias trade off (Hastie, Tibshirani and Friedman, 2009). Advantages of feature selection methods such as lasso and MARS modelling over conventional regression have been shown by Kuhn and Johnson (2013), who demonstrate a progressive increase in RMSE when adding non-informative predictor variables for conventional model types such as linear regression, in contrast to algorithms such as MARS (amongst others) where the addition of non-informative variables had no significant impact on model performance.

One solution that has been specifically designed with the p>>n problem in mind are shrinkage methods such as the elastic net (Zou and Hastie, 2005). A major incentive to develop these algorithms was the potential to explore large numbers (many thousands) of variables on small numbers of samples, specifically referencing the usefulness of the elastic net algorithm in datasets such as genetic datasets with many thousands of predictor variables (often being highly correlated) and <100 observations. Shrinkage based regression is likely to be superior to conventional regression approaches both in terms of prediction and interpretation in these types of datasets (Zou and Hastie, 2005), and represents a useful approach when analysing high dimensional data.

1.7. Shrinkage methods

Whilst the selection of a subset of variables is often desirable in veterinary epidemiological research, there is a risk of high variance due to the binary nature of retention/discarding variables. Shrinkage methods are methods that impose penalties on coefficients to shrink them either towards, or to, zero. Consequently, they don't suffer as strongly from the issue of high variability, as they are more likely to provide a similar subset of variables and coefficients during subsequent resamples than conventional stepwise methods, where the subset of variables may differ substantially in response to slight alterations in the data (Hastie, Tibshirani and Friedman, 2009).

Ridge regression aims to shrink regression coefficients to limit their size. Contrary to a linear regression approach where variables might be removed when a large positive coefficient is paired with a large negative coefficient of a correlated variable, ridge regression alleviates this by imposing a size constraint on coefficients as follows

$$\text{RSS} + \lambda \sum_{j=1}^{p} \beta_j^2,$$

where $\lambda \sum \beta_j^2$ represents the shrinkage penalty and $\lambda \geq 0$ a tuning parameter that controls the magnitude of impact this penalty has on shrinking estimates of β_j towards zero. A disadvantage to this approach, however, is the retention of all variables within the final model, due to the shrinkage towards, rather than to, zero. The lasso algorithm provides a solution to this problem, by imposing a relatively severe form of feature selection, and whilst this shrinkage method is relatively similar to ridge regression it differs by causing a subset of coefficients to be exactly zero as

$$\text{RSS} + \lambda \sum_{j=1}^{p} |\beta_j|,$$

which uses an ℓ_1 penalty, as opposed to the ℓ_2 penalty utilised by ridge regression. Where there are strong correlations between variables, the lasso might include a set of strong, but uncorrelated variables, whereas the ridge penalty might shrink coefficients of correlated variables towards each other (Hastie, Tibshirani and Friedman, 2009). The elastic net penalty is a compromise between ridge and lasso. The elastic net function to be minimised might take the form

$$\sum_{i=1}^{n}(y_i - \hat{y}_i)^2 + \lambda_1 \sum_{j=1}^{p} \beta_j^2 + \lambda_2 \sum_{j=1}^{p} |\beta_j|,$$

where y_i and $\hat{y}_i$ represent the observed and predicted outcome for the *i*th observation of *n* observations respectively and *j* a predictor variable of *p* predictor variables. λ_1 and λ_2 represent parameters to be tuned affecting the penalty imposed on ridge and lasso regression coefficients respectively. The extent of penalisation might be altered by providing various options for λ which will progressively decrease the variability at the expense of introducing a relatively low level of bias. The optimal model can subsequently be selected through analysis of model performance (such as minimising MSE or RMSE) at various values of λ.

Shrinkage methods such as lasso and ridge (and consequently elastic net as a product of these) (Zou and Hastie, 2005) have been shown to be applicable and advantageous when covariates are highly correlated, and in particular are able to control for potential confounding between variables where conventional methods fail (Greenland, 2007). In addition, when determining best subsets of variables there is a computational advantage to regularisation methods which only require the fitting of a single model to determining the best subset of p variables, compared with 2^p models required for stepwise selection, which can quickly become infeasible in a high dimensional setting (James *et al.*, 2017).

1.8. Model assessment

Alongside the flaws of conventional regression techniques in a high dimensional setting such as erroneously removing the effect of many variables, there also remains an issue of how to evaluate the "best" model. Common metrics for characterising a model's predictive capabilities are the root mean squared error (RMSE) or the mean squared error (MSE) both being functions of the model residuals to interpret how far the residuals are from zero (Kuhn and Johnson, 2013).

Conventional regression models are often analysed in terms of an "internal fit", meaning that the data used to build a given model is also used to determine its performance, often resulting in overly optimistic performance characteristics, and the ability to generalise to the

wider population is unknown. A more common approach within the field of statistical learning is the assessment and validation of models on data separate to that which it is trained (Hastie, Tibshirani and Friedman, 2009), since future values of variables are unlikely to coincide with their training set values. In its simplest form, this might involve removing a proportion of the observations within a dataset for testing purposes only. This allows model performance to be "trained" on a given dataset, and "tested" on separate data not involved within the model building process. Losing observations, particularly in relatively small datasets, however, is often undesirable and there are several other methods of assessing model performance using a similar approach without loss of data.

Cross-validation is often utilised as a robust method of analysing model performance by repeatedly building and testing a given model on different portions of the dataset. For K-fold validation, the data would be split into K parts (or "folds"), where the model is trained on all but one of the K folds. Common choices of K are 5 or 10, and the case where K = N is known as leave-one-out cross validation. This process can be repeated (typically 10 times), to provide robust analysis of model performance (James *et al.*, 2017).

It is crucial that cross-validation be performed as the very first step in the modelling procedure, as selection bias is likely if performed after filtering to achieve a subset of variables. An example of this is presented by Kuhn and Johnson (2013), where the authors discuss the results of a study utilising recursive feature elimination to identify a subset of variables, and only subsequently calculated performance of a support vector machine model using this subset of variables using cross validation (Guyon *et al.*, 2002) to achieve an impressive accuracy of 100%. This overly optimistic performance was demonstrated by Ambroise and McLachlan (2002) by achieving the same model performance, despite having permuted the predictor values to be completely non-informative. Whilst cross-validation represents a robust method of estimating model performance, it is essential that it is performed in advance of any variable selection methods being applied.

The bootstrap (Efron and Tibshirani, 1994) is another option for the analysis and validation of model performance. The basic principle of the bootstrap is to draw new datasets from the original dataset by randomly sampling the observations with replacement B times. Where for example B=1,000 would result in 1,000 datasets each containing various observations from the original dataset, with many observations either being repeated or entirely missing from a given new dataset. By sampling with replacement, each observation will be present at least once in ~0.632n (~63.2%) of bootstrap samples (Borra and Di Ciaccio, 2010). The effect of a single outlying value is thereby dramatically reduced, as in almost ~36.8% of models it cannot influence the model in any way at all. Bootstrapping procedures can ameliorate

issues of excessive leverage often encountered in conventional approaches, and the bootstrap approach been shown to provide robust estimates of model performance (Breiman, 1996).

1.9. An introduction to stability

Stability selection was initially developed for variable selection in a high dimensional setting (Meinshausen and Bühlmann, 2010), and has been demonstrated to reduce the likelihood of over-fitting in a veterinary epidemiological setting (Lima et al., 2020). Stability selection involves sampling the data using conventional bootstrapping (Efron and Tibshirani, 1994), and subsequently calculating the percentage of times that a given variable is selected by a given model across all bootstrap resamples, known as the stability. The distributions of coefficients for each variable can also be reported, and a bootstrap p-value might be calculated as the smallest proportion of non-zero coefficient values on one side of zero.

A stability selection protocol might be performed as follows:

1. Bootstrap sample (random sampling with replacement) from dataset
2. Fit a given model on the bootstrapped sample and save coefficient results for selected variables
3. Repeat 1,000 times
4. Calculate stability percentage as the proportion of the 1,000 samples where a given variable was selected and explore coefficient distributions across resamples

This is a particularly useful approach in an epidemiological research setting, as variables might be analysed in terms of not only the size of the effect they might have on an outcome (the mean coefficient), but also the likely reproducibility of the variable effect in the target population (stability). It is critical for veterinary advisors to have the ability to target a small number of management interventions likely to have the maximum gain, in the majority of cases, and stability selection approaches provide a robust method of achieving this, particularly in conjunction with regularisation techniques.

1.9.1. An empiric stability selection threshold

In conventional modelling, a variable where $p<0.05$ is often deemed as “significant” and all other variables are excluded from the model. It has been recognised within the scientific community that a statistically non-significant result does not prove a null hypothesis, nor does a statistically significant result prove an alternate hypothesis (Amrhein, Greenland and McShane, 2019). The dichotomisation of variables as either “significant” or “non-significant” is an issue whether from the somewhat arbitrary classification based on p-values or by using other methods such as stability selection.

In a discussion of the stability threshold in the original stability selection research produced by Meinshausen and Bühlmann (2010), a precise "optimum" threshold was not described, and of course will depend largely on the problem/dataset being investigated. Meinshausen and Bühlmann did however demonstrate that by increasing the stability threshold, the false positive rate decreases, albeit at the expense of an increasing false negative rate. Currently, there is no statistical method to identify an optimal stability threshold, and a 60-90% threshold is often chosen with the aim of minimising both false negatives and false positives (Meinshausen and Bühlmann, 2010).

An empiric threshold for stability selection has recently been proposed (Green, Lima and Hyde, 2021) and has been developed in parallel to the statistical challenges encountered during this thesis. Rather than having a fixed arbitrary value, this threshold is flexible, and calculated from the background variation in the dataset. A stability selection threshold can be determined through the random permutation of outcome variables to sever the relationship between outcome and explanatory variables in order to calculate a null threshold using an empirical cumulative distribution function. This 'null threshold' can then be utilised as a threshold for variable selection as follows:

1. Randomly permute the outcome variable to remove relationships between outcome and explanatory variables, repeated ≥5 times.
2. Calculate stability values for all explanatory variables across 20 bootstrap samples for each of the 5 permuted sets.
3. Determine null threshold through use of an empirical cumulative distribution function to determine the mean highest value across all 5 permutation repeats.
4. Calculate stability values for all explanatory variables across 100 bootstrap repeats on the full dataset (i.e., without permutation).
5. Variables with stability values in excess of the null threshold are considered to be truly associated with the outcome.

This proposed method has the advantage of being applicable to any model type, and any dataset. Variables with a stability in excess of the null threshold have been shown to be extremely unlikely to be false positives (Green, Lima and Hyde, 2021) and the chances of overfitting are far reduced in comparison with conventional modelling approaches relying on a single model fit with an arbitrary p-value as a threshold for "significance".

1.10. Triangulation

Given the range of potential models to choose it can be challenging to select the "correct" model type for a given scenario, and this will depend on the application of the model, with

certain models performing better in certain conditions. This is similar in some respects to the "no free lunch theorem" which pertains to model tuning and describes the point where the improvement in one value will be to the detriment of another (Wolpert and Macready, 1996). The availability of algorithms and both the flexibility and complexity of data analysis workflows have increased, and the analysis of magnetic resonance imaging data by 70 independent teams has shown that no team chose the same workflow, and sizeable variation in the result of the hypothesis tests (Botvinik-Nezer *et al.*, 2020). Given the enormous range of techniques available and in use, the robust determination of which approach or model is "correct" is becoming increasingly challenging and a single model approach is unlikely to provide a uniquely "correct" solution in epidemiological studies. Indeed, different types of model will tend to introduce different types of bias.

It has also been shown in the veterinary field that different methods of variable selection can result in significant differences in the variables selected (Lima *et al.*, 2020). Along with calls to discard the often used "statistical significance", it has been suggested that uncertainty in research be explored from multiple angles (Nature editorial, 2019). Triangulation represents a potential solution (Munafò and Davey Smith, 2018) through the combination of multiple models or techniques to increase confidence in the outcomes, and the principles of triangulation might be applied to epidemiological studies, where a higher degree of confidence might be granted to variables being identified as causal across multiple model types.

Approaches for combining multiple model types along with selection stability have been proposed and combined methods using stability selection with multiple models have been shown to generally perform as well or better than individual methods in simulated datasets (Lima, Hyde and Green, 2021). Once coefficient distributions and stability values have been calculated across multiple methods, a triangulated result might be obtained by aggregating bootstrap matrices from the multiple methods and calculating stability values across this "combined" matrix as follows:

1. Bootstrap sample (sampling with replacement) from dataset
2. Fit a given selection of models on the bootstrapped sample and save coefficient results for selected variables.
3. Repeat 1,000 times
4. Aggregate stability matrices from all model types
5. Calculate stability percentage as the proportion of the samples where a given variable was selected across all model types.

The principles of triangulation are often furthered by the selection of relatively dissimilar model types, and our confidence in the degree of association between a variable and outcome might be increased if the variable is selected across methods with fundamental differences. In addition to providing a stability percentage through this approach, an empiric threshold for stability selection can also be calculated across multiple model types as previously described.

1.11. Other methods

There are many algorithms that might be chosen to approach a dataset with high dimensionality. There are a multitude of other algorithms available within the field of statistical learning. Principal component analysis (Wold, Esbensen and Geladi, 1987) has historically been used to find the best subset of variables, aiming to reduce the dimensionality of the dataset. Although a fewer number of features is obtained by the end, this is not a true feature selection method as each principal component is a combination of the original features (James *et al.*, 2017). The random forest (Breiman, 2001) has also received particular attention as a powerful classification algorithm (Fernández-Delgado *et al.*, 2014), and whilst these methods are certainly powerful in certain circumstances, they are relatively "black box" methods, where there are not easily discernible coefficients available for specific variables that would be desirable within an epidemiological study. Visualisation of the main effects within "black box" methods are available in the form of partial dependence plots and accumulated local effects (ALE) plots etc (Apley and Zhu, 2016), however direct interpretation is often not as straightforward as with approaches such as regularised regression.

One theory worth considering is the "bet on sparsity principle" which suggests that since no procedure does well in a dense problem (with a large number of causal variables) it is better to assume that the true solution is relatively sparse (where only a relatively small number of variables are truly causal) and select an algorithm that performs well in this setting (Hastie, Tibshirani and Wainwright, 2015).

1.12. Aims of the thesis

The aims of this thesis were to identify key factors associated with health and productivity of pre-weaned calves, using statistical learning techniques, and subsequently to test factors identified as important in a randomised controlled trial.

The aims of Chapter 2 were to provide a benchmark of calf mortality across GB and analyse factors associated with mortality levels at a national level using multivariate adaptive regression splines. To determine factors associated with growth rates on dairy farms, 60

farms across GB were recruited as described in Chapter 3, and the aims of this chapter were to identify farm management factors associated with growth, morbidity and mortality outcomes using shrinkage methods such as elastic net regression in conjunction with stability selection. Factors associated with colostrum bacterial levels were also analysed using stability selection using a stepwise selection approach within a mixed model framework described in Chapter 4 and the aims of this chapter were to provide the first benchmark of colostrum bacteriology levels in GB and identify factors associated with high bacterial levels in order to provide practical and efficacious recommendations for veterinarians and farmers. The findings from these early chapters were utilised to create a calf health plan, and the aim of Chapter 5 was to test this calf health plan as a randomised controlled trial. To further explore the effects of environmental temperature, the use of heat lamps and calf jackets were also explored as a randomised controlled trial in Chapter 6, where the aims were to assess the effects of calf jacket and/or supplementary heat on growth rates in preweaned calves and evaluate associations between environmental temperature and growth utilising data from multiple sources within a Bayesian framework.

A key outcome from this thesis was the creation of vet and farmer focussed online tools, so that the outcomes from this research might be translated into clinical practice. Similarly, the developments in statistical techniques across this thesis would be more accessible to researchers if streamlined within an easily accessible package. Consequently, the final aims of this thesis were the creation of a freely available herd health toolkit providing practical management recommendations based on the research findings, and the creation of an R package providing researchers with the statistical tools necessary to perform selection stability in conjunction with multiple model types in epidemiological datasets.

This research was conducted following ethical approval from the University of Nottingham Committee for Animal Research and Ethics (2119 17091).

Chapter 2 Quantitative analysis of calf mortality in Great Britain

This chapter outlines current calf mortality rates in Great Britain, and potential factors associated with mortality rates at a national level are discussed. A close derivative of this chapter has been published in the Journal of Dairy Science (Hyde *et al.*, 2020).

2.1. Introduction

Neonatal mortality (defined as 1d of age – weaning, Compton *et al.*, 2017) represents a significant loss to the British cattle industry. Calf management is critical in rearing productive dairy cows (Hultgren and Svensson, 2009), and represents a significant economic outlay, accounting for around 20% of total dairy farm expenditure (Gabler *et al*., 2000) with costs in Great Britain estimated at around £1819 per animal (Boulton *et al*., 2017). A study following 1097 calves from 19 farms in England suggest 15% of liveborn dairy heifers die before reaching their first lactation (Brickell *et al.*, 2009), with the cost of heifer mortality representing around £139 per animal when spread across surviving animals (Boulton *et al*., 2017). Effective calf management is also crucial for efficient beef production (Mõtus *et al.*, 2017), a significant industry for the UK, being the third largest producer of beef in Europe (DEFRA, 2018).

Neonatal mortality not only represents an economic loss, but also delays genetic progress by providing fewer replacements for voluntary culling (Raboisson *et al.*, 2013). Mortality has also been explored as a marker for farm welfare surveillance and has been suggested as an indicator of overall health on cattle farms (Ortiz-Pelaez *et al.*, 2008; von Keyserlingk *et al.*, 2009). The effective management of neonatal calves is essential for survival, welfare, and productivity (Renaud *et al.*, 2018), and whilst mortality in calves is unlikely to be entirely eradicated, it should be a goal to reduce it by as much as possible (Santman-Berends *et al.*, 2014)

In order to prevent disease and reduce mortality, it is essential to understand the incidence, prevalence, distribution and key factors that influence disease variability; this is the basis of epidemiology and is recognized as a first step in disease control (Dohoo, Martin and Stryhn, 2009). National bodies in Great Britain (GB) have expressed concern over youngstock health and welfare, identifying calf survival as a priority (CHAWG, 2017, 2018b). However, there are currently no national data being published and therefore the extent of the problem remains largely unknown. A clear understanding of patterns of calf mortality on a national scale would inform stakeholders of whether and where to allocate resources both for

knowledge exchange and further research. Furthermore, quantification of risk between groups (for example, beef or dairy calves, male or female calves and specific times of year) would allow targeting of resources towards high risk populations and time periods.

The use of national level data for epidemiological studies has been called for to help develop methods of reducing morbidity and mortality (Santman-Berends *et al.*, 2014; Veldhuis *et al.*, 2016) but rates of calf mortality in GB have not been evaluated nationally since 2007 (Gates, 2013). Whilst keepers of bovine animals in GB must register births, deaths, and movements of their animals through the Cattle Tracing System, the data are not used to routinely report national incidence rates of calf mortality.

The aim of this study was to quantify the temporal incidence rate, distributional features and factors affecting variation in mortality rates in calves in Great Britain since 2011. The purpose was to provide information to national stakeholder groups to inform resource allocation for both for knowledge exchange and future research.

2.2. Materials and methods

Birth and death data from 2010-2019 were requested from British Cattle Movement Services (BCMS). Data were only available at county level, and included the number of births for each county, country, breed, sex, month, and year as an aggregated figure. Death data were provided in a similar format, however excluded county level information, and contained the number of deaths for each age at death (months), premises of death (i.e., "on farm" or "slaughterhouse"), country, sex, breed, month, and year. "On farm" deaths included animals that died on farm, and "slaughterhouse" deaths included any animals that were transported to a slaughterhouse for slaughter. These datasets were combined to allow calculation of estimated mortality rates for defined groups.

Births and deaths (of any age) were filtered to be after 2010 and before 2019. The premises of death comprised five categories: slaughterhouse, farm, market, hunt/knackers (colloquial terms for fallen stock removal enterprises) and other. The vast majority of deaths (99.97%) were reported on-farm or at a slaughterhouse. Given that less than 0.03% of deaths were categorized as market, hunt/knackers or other it was decided to exclude these from the dataset. Breeds were categorized as either "Dairy" or "Non-dairy" (i.e., beef, including beef cross dairy) according to BCMS categories. A small proportion (<0.05%) of animals were non-cattle (i.e., Bison, Yak and Water buffalo) and were removed from the dataset.

Cumulative mortality rates were calculated as a percentage of animals born in a specific month subsequently dying within a defined time period. For example, of 100 calves born in January, two calves dying in January at 0-1 months of age, two dying in February at 1-2

months of age, and one dying in March at 2-3 months of age would result in a 0-3 month mortality rate of 5%. Country and county were excluded from analysis to avoid the potentially erroneous assumption of zero cross border transport between birth and death (e.g., the assumption that calves that die in Scotland were also born in Scotland).

British cattle must be dual tagged within 20d of birth, and all births and deaths must legally be registered through BCMS. There is a requirement that deaths that occur prior to tagging also be recorded within the holding register, and inspectors visit a proportion of UK farms (at least 3% of holdings annually) to validate identification and record keeping protocols (UK Government, 2014). Whilst it is unlikely that many stillborn (0-24hour mortality) calves will be included within registered deaths, there remains uncertainty as to the current stillborn rate in GB.

Descriptive and statistical analyses were conducted in R (R Core Team, 2017). On farm mortality was treated separately to slaughterhouse deaths, and descriptive graphical analysis was conducted by breed, sex, age, month and temperature. Linear regression methods were employed to provide insight into potentially influential factors associated with calf mortality at 0-3 months of age. These factors included the breed, sex, month of birth and Met Office meteorological data (mean, minimum and maximum monthly temperatures across the UK). To explore interactions between variables and non-linearities within the data, multivariate adaptive regression spline models (Friedman, 1991) were employed using the *earth* (Milborrow, 2019) and *caret* (Kuhn. *et al.*, 2018) *R* packages. Interactions up to order 3 were tested and the maximum number of terms available to the model was explored and optimized using a dense grid of values between 2 and 25 in increments of 1. Ten -fold cross-validation repeated 10 times was used to identify the optimal value of these tuning parameters; optimization was based on minimizing model mean absolute error (MAE). Final model covariates were therefore selected based on minimizing the cross-validation model MAE; covariate selection is an integral part of the MARS procedure (Friedman, 1991). An evaluation of model fit and the extent of over-fitting was assessed by a comparison of MAE and R^2 computed from the final model based on the full dataset ('internal fit') and computed from 10-fold cross validation ('cv-fit'). Residuals were examined to ensure model fit, by examining fitted values against residuals to ensure the model was not over-, or under-predicting mortality rates, particularly at the extremities of fitted values. Human mortality was also available stratified by sex and month of death through the office of national statistics and was included in comparison to calf mortality (ONS, 2020).

To estimate the effect of optimizing environmental conditions on calf mortality, covariates in the final model were used to predict how mortality might change with different environmental temperatures or different months of birth.

2.3. Results

At the time of analysis, the cattle population in the UK was around 9.6 million (UK Government, 2018). The number of births and deaths registered between 2011-2018 across GB were 21.2 and 21.6 million respectively, of which 18.3 million and 3.3 million deaths were at slaughterhouse and farm respectively. Of the total on-farm deaths 1.8 million occurred before 24 months of age (54%, Figure 2-1), and 819,703 (25%) happened within the first 3 months of age.

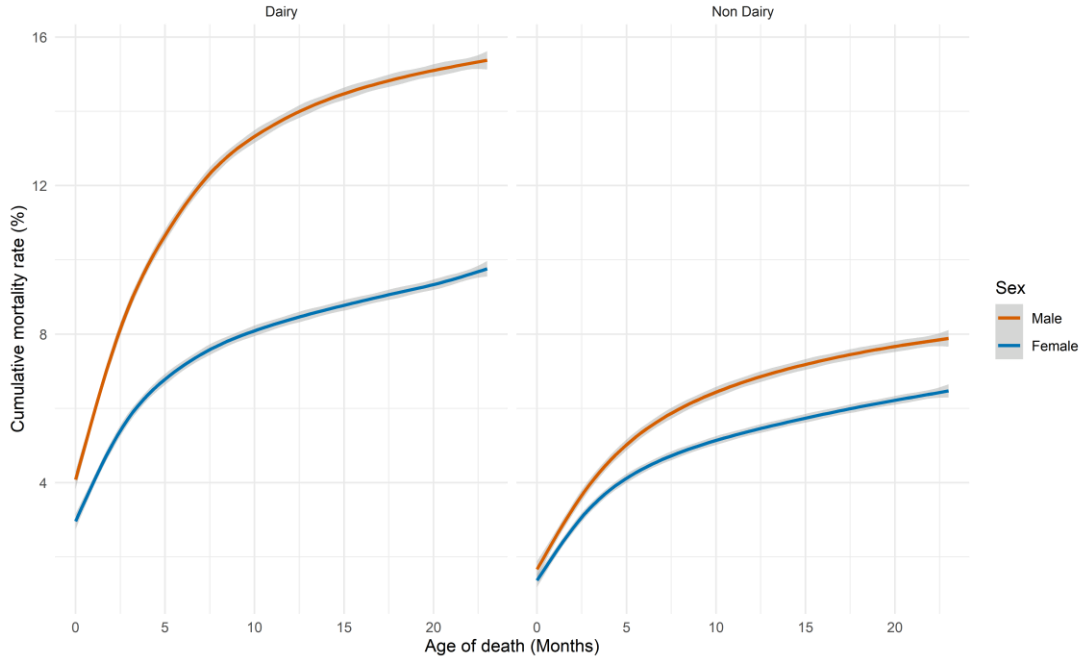


Figure 2-1: Cumulative on-farm mortality rates by age, sex and breed type. 95% confidence interval as grey shading.

Of the 818,845 dairy cattle that died on farm before 24 months of age, 409,612 (50%) died within the first 3 months of age, and of the 956,293 non-dairy cattle that died on farm before 24 months of age, 410,091 (43%) died within 3 months of age. Of the 18.3 million deaths that were recorded at slaughterhouses, 644,848 (3.5%) were before 3 months of age. The 0-3 month death rate at slaughterhouses (and excluding any on farm mortality) was 0.49%, 0.21% and 0.34% for dairy females, non-dairy females and non-dairy males respectively, and was 19.94% for dairy males.

The on-farm (excluding any slaughterhouse deaths) mortality rate was 3.87% by 3 months of age, and was higher for male than female calves, with male calves experiencing a mortality rate of 4.32%, compared with 3.45% for female calves. Dairy calves experienced higher mortality rates than non-dairy calves within 3 months of age, with 6.00% and 2.86% mortality rates respectively. Male dairy calves had the highest on farm mortality rate within 3 months of 7.37% compared with a mortality rate of 4.96% for female dairy calves. Female non-dairy calves had the lowest mortality rate within 3 months of 2.61% compared with a mortality rate of 3.10% for male non-dairy calves. On-farm mortality rates remained relatively stable over time (Figure 2-2).

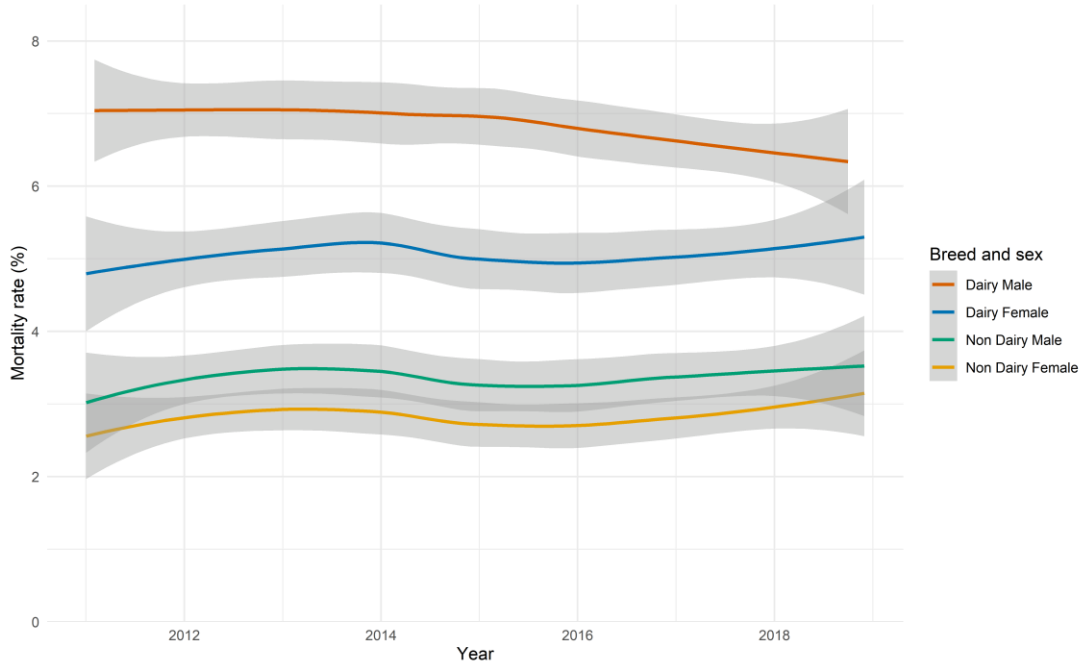


Figure 2-2: On-farm mortality rate 0-3months of age (%) by year breed type and sex from 2011-2018. 95% confidence interval as grey shading.

Male dairy calves appeared to be the only category of animal being routinely sent for slaughter at 0-3 months of age, and this appeared to show an upward trend over time, from 17.40% in 2011 to 26.16% in 2018 (Figure 2-3).

There appeared to be a strong seasonal component to 0-3 month on farm mortality rates across both breed types and sexes (Figure 2-4), with dairy calves having a lower 0-3 month mortality rate during summer, with a rate of 6.61%, 6.11%, 4.79% and 6.74% for dairy calves born during winter, spring, summer and autumn respectively. Similarly, non-dairy calves had a lower mortality rate when born during summer, and also had a lower rate during spring, with the highest rate being autumn (winter: 2.88%, spring; 2.56%, summer: 2.56% and autumn: 3.98%).

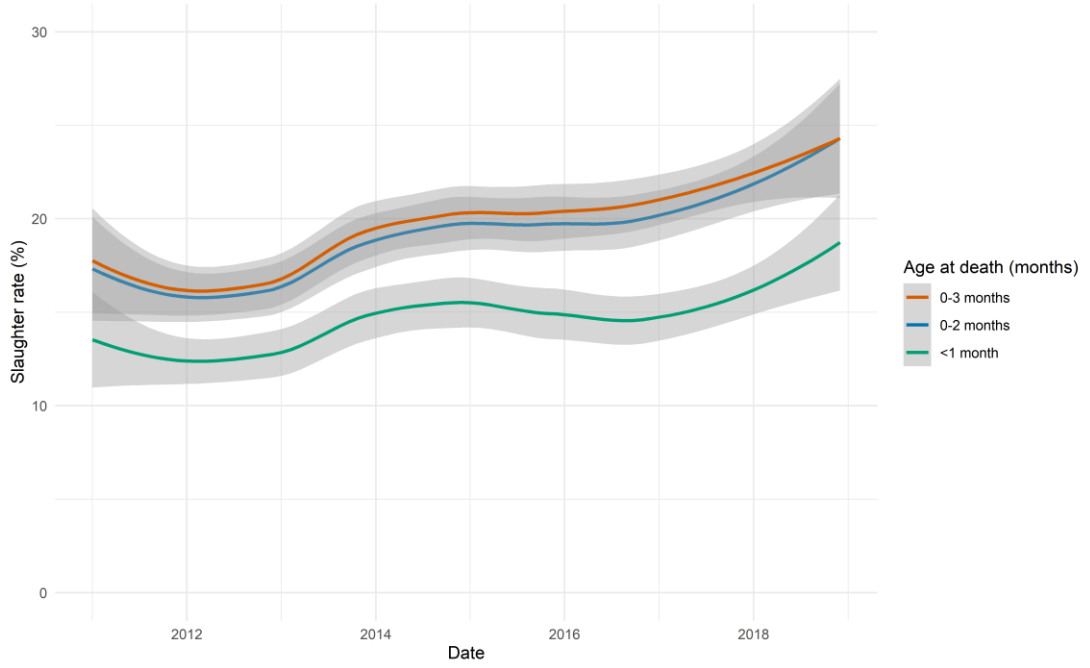


Figure 2-3: Slaughter rate over time within 1 month, 0-2 months and 0-3 months of age. 95% confidence interval as grey shading.

Calf and human mortality rates were relatively similar both in terms of seasonality and sex and are shown in Figure 2-5. There also appears to be a relatively strong association between temperature and mortality rates, independent of the month of birth, and trends appear similar for both male, female, beef and dairy calves (Figure 2-6). Visual analysis of this association suggests that increasing temperature is negatively associated with mortality rates across breed types and sexes, but that the strength of this association decreases during summer-autumn (May-September) compared with winter and spring (October-February).

Results of the final MARS model are provided in Table 2-1. Both sex and breed type were associated with differences in mortality rate, with an increased rate for male calves, and decreased rate for beef calves respectively. Month of birth had an effect on mortality rate, and the mortality rate in the first 3 months of life increased for calves born in December by +1.1%. Whilst mortality rate was reduced for calves born during February (-1.4%), there was an interaction between breed type and month of birth (Feb*Non-dairy) of +1.0%, resulting in a predicted mortality rate change of -0.4% for Non-dairy calves born in February (as opposed to dairy calves born in February which had a predicted rate of –1.4%). Similarly, dairy calves born in November had a mortality rate change of +0.3%, compared to +0.9% for Non-dairy calves (the interaction effect of Nov*Non-dairy = +0.6%). There were interactions between month and breed type for February, March, October and November, and interactions between month and sex for January and August indicating that these subsets of animals at these specific times had different predicted risks of mortality.

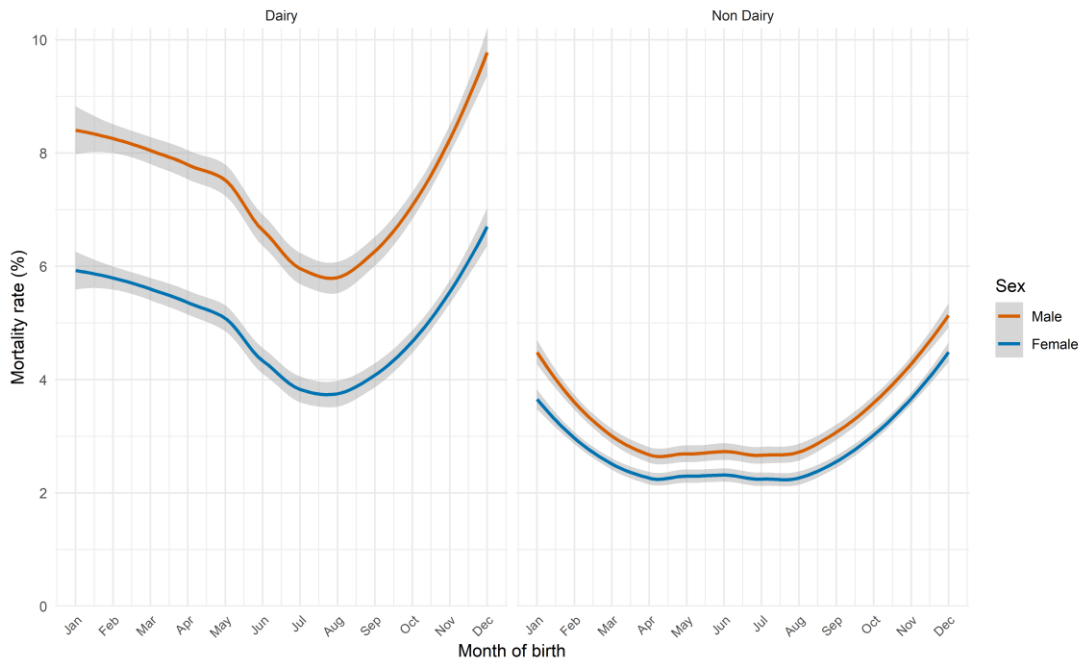


Figure 2-4: Seasonal patterns in on-farm 0-3 month mortality rates by breed type and sex. 95% confidence interval as grey shading.

MARS models identified a hinge point within the mean monthly temperature variable at 4.8℃, and at 9.6℃ where an interaction effect with Non-dairy (beef) calves was included. This indicated that reduced mean monthly temperatures were associated with increased calf mortality regardless of month, and this association was slightly stronger below 4.8℃. An interaction term for non-dairy breed type and temperature was present; Non-dairy*h(Mean temperature – 9.6). This interaction term coefficient indicated a change in mortality rate for non-dairy calves (as denoted by *non-dairy*h*) for each 1℃ above 9.6℃ (as denoted by *(mean temperature – 9.6)*) of -0.2%, effectively neutralizing the 0.2% decrease in mortality above 4.8% suggested by the term h(4.8 – Mean temperature). In short, this indicated that the mortality of non-dairy calves increased by 0.2% for every 1℃ decrease in temperature, but that this effect was limited to below 9.6℃ only, and there was minimal effect of temperature on the mortality rate of non-dairy calves above this point.

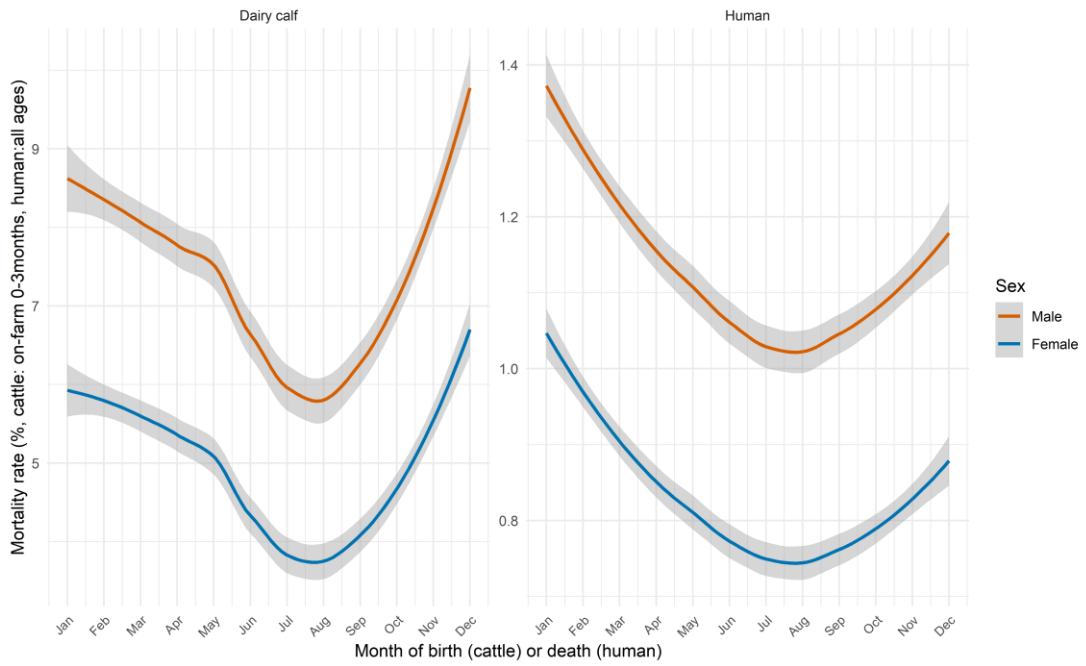


Figure 2-5: Similarities between dairy calf and human mortality rates by seasonality and sex between 2011-2018.

Analysis of model fit showed an R^2 value of 95.77%, RMSE of 0.44 and mean absolute error (MAE) of 0.34 when using 10-fold cross validation, and an R^2 value of 96.24%, RMSE of 0.40 and (MAE) of 0.31 when using internal fit, indicating there were no signs of model overfitting. Optimal model parameters were a degree of 2 interactions, and the number of terms for inclusion (*nprune*) set at 16.

Variable	Coefficient
Intercept	6.0
Breed type (Non-Dairy)	-2.7
Sex (Male)	2.4
h(Mean temperature -4.8)	-0.2
h(4.8- Mean temperature)	0.2
Sex (Male) * Breed type (Non Dairy)	-1.9
Month (Mar) * Breed type (Non Dairy)	-1.2
Month (Feb)	-1.4
Breed type (Non Dairy) * h(Mean temperature-9.6)	0.2
Month (Dec)	1.1
Month (Nov)	0.3
Month (Feb) * Breed type (Non Dairy)	1.0
Month (Oct) * Breed type (Non Dairy)	0.7
Month (Jan) * Sex (Male)	0.6
Month (Nov) * Breed type (Non Dairy)	0.6
Month (Aug) * Sex (Male)	-0.4

Table 2-1: Results from multivariate adaptive regression splines (MARS) model. "h" denotes hinge point.

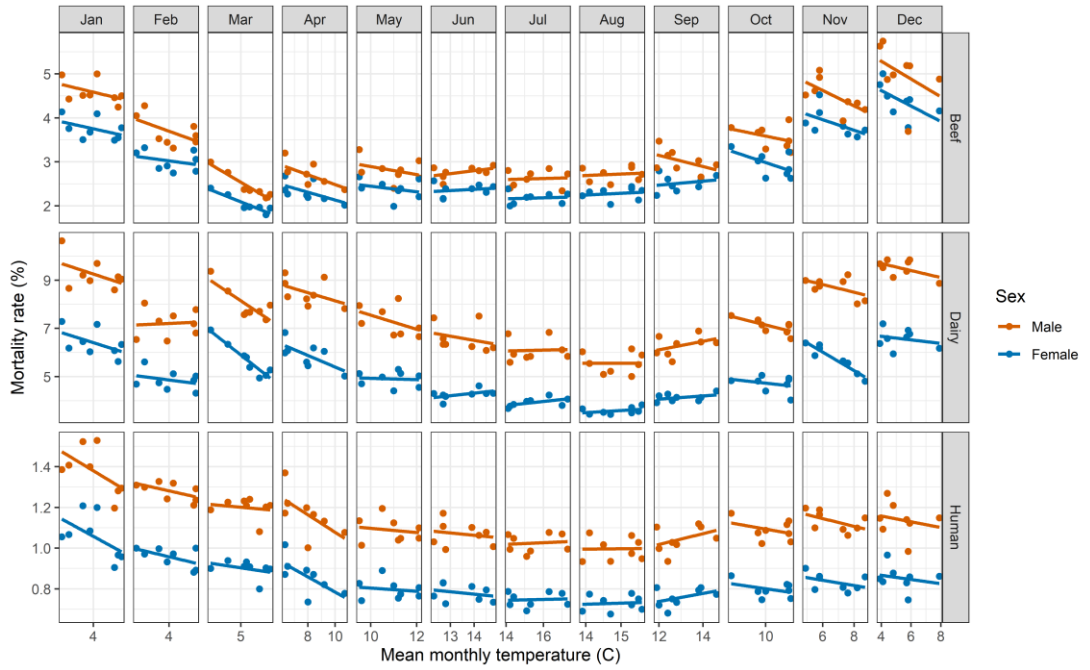


Figure 2-6: Mortality rate at by mean monthly temperature (C) in a given month by sex for beef and dairy calves (at 0-3 months) and humans (all ages). Months Jan-Dec are shown in panels left to right, with beef breeds in top panels, dairy breeds middle, and human mortality below.

To examine the effect of environmental conditions on calf mortality the final model was used to predict mortality given optimal month and environmental temperature. Calves born in February were shown to have the lowest mortality rate independent of temperature, and increased mean monthly environmental temperature was shown to decrease mortality rates at 0-3months of age independent of month (the maximum monthly mean temperature recorded was 17.3℃).

Predictions of mortality were made by assuming all calves were born in February and with the environmental temperature set constantly at 17.3℃. This resulted in an estimated total reduction of 300,570 deaths at 0-3 months of age over the period 2011-2018, equating to a mean reduction of 37,571 fewer deaths per year.

2.4. Discussion

This research represents one of the largest calf mortality datasets ever reported and suggests minimal change in GB calf mortality rates between 2011 and 2018. The results indicate that environmental temperature, time of year, sex, and breed type are strong predictors of mortality rate, and account for around 96% of the total variation in on-farm mortality at national level. Importantly, it appears that relatively low mortality rates are achievable at specific times of year, in certain groups of animals and in relatively warm temperatures. For example, in the lowest risk groups for both dairy and non-dairy breed types (female calves

born in February) a mortality rate of <2% would be expected at temperatures of 17.3^{0}C (the maximum monthly temperature recorded). If the environmental conditions provided for these groups of animals could be identified and replicated through improvements in housing management, a reasonable national target for the British cattle industry could be to reduce overall 0-3 month calf mortality to <2%. To achieve this, further research into and understanding of these specific environmental conditions is required; these areas are discussed below.

The effect of environmental temperature and month of birth appear to play a significant role in neonatal mortality rates. Reductions in mortality during summer has been previously reported in both dairy (Lombard *et al.*, 2007; Raboisson *et al.*, 2013) and beef calves (Mõtus *et al.*, 2017), and colder temperatures have been suggested as an important factor in mortality rates, with temperatures of ≥10 °C being associated with lower mortality (Pannwitz, 2015). This is reinforced with more recent studies reporting an increased risk of mortality in cold conditions using national data from the Netherlands (Egberts *et al.*, 2019). Colder weather may also have a negative effect on calf vigour, which may subsequently affect the transfer of passive immunity (Olson *et al*., 1980; Robison *et al*., 1988); an essential component in reducing morbidity and mortality (Godden, 2008; Cuttance *et al.*, 2018). There may also be an effect of increased infection pressure during housing through winter, which might subsequently increase the risk of morbidity and mortality (Raboisson *et al.*, 2013). The large effects of month and temperature suggest a substantial environmental component to the risk of mortality; if calf housing was able to replicate optimal environmental conditions all year round, major reductions in mortality could result. The potential mean annual reduction in mortality of 37,571 fewer deaths identified in this study as attributable to sub-optimal environmental conditions, represents a potential economic saving of around £11.6 million per year when using an estimate of £310 per calf death (Kossaibati and Esslemont, 1997) as well as having obvious welfare implications. The impact of environmental conditions on calf mortality rates in GB should certainly be the subject of future research.

Higher neonatal mortality rates in males compared with females has been previously reported (Raboisson *et al.*, 2013; Pannwitz, 2015; Cuttance *et al.*, 2017), potentially related to the higher mortality associated with concomitant dystocia (Johanson *et al.*, 2011), particularly for breeds with heavier birth weights (Gundelach *et al.*, 2009; Linden *et al*., 2009). Male dairy calves are also often regarded as less economically valuable as heifer calves, and thus may not receive the same standard of care (Renaud *et al*., 2017), potentially resulting in higher morbidity and mortality rates. Variations in neonatal mortality rates between breed types has previously been reported and is possibly due to management of the

breeds rather than the breed themselves (Raboisson *et al.*, 2013). Whilst mortality risk might be hypothesised to be higher in beef crossed calves due to increased dystocia (Raboisson *et al.*, 2013), this study suggests that dairy animals are at increased risk of mortality. This has been previously reported in a study of 1.3 million Slovenian calves reporting a 2-30d mortality rate of 2.68% and indicating calves from Holstein Friesian dams having higher mortality rates than calves from other breeds, with herd size and calving season also being important factors influencing mortality rate (Voljč *et al.*, 2017). Results from the current study suggest there is an urgent need for additional research to identify strategies to reduce mortality rates in male dairy calves in particular.

The increasing trend of calves being sent for slaughter by 3 months of age has not been previously reported in GB, and whilst it is not possible to identify reasons for this increase in this study, it is likely that there are a range of social and economic factors involved. An increase in the number of male dairy calves entering the beef chain rose by 59% from 2006 to 2015, and recent estimates indicate 81% of all male dairy calves in 2015 were reared for beef in GB (CHAWG, 2018a). The fate of male dairy calves is an important issue for the industry (Renaud *et al.*, 2018), and the increase in male dairy calves entering the beef chain may, in part, be due to this increased rate of early slaughter. There is a slight reduction in on-farm mortality of male dairy calves at 0-3 months of age, however this may also be due to the increased rate of early slaughter of male dairy calves.

Patterns in mortality rates for both dairy calves and humans were remarkably similar, although on a different scale as shown in Figure 2-5. Increases in mortality rates in the human population have been previously reported to be associated with temperature in England and Wales (Brown, Fearn and Wells, 2010), showing similarities to the findings of the current study.

Previous on farm mortality estimates for GB calves are relatively sparse, with previous estimates from national CTS data suggesting an on-farm 0-6 month mortality rate of 2.47% for beef and 7.42% for dairy calves born in 2007 (Gates, 2013), compared with 4.15% and 8.31% 0-6 month mortality rates found in the current study for beef and dairy respectively. Research from 11 farms in the south-east of England in 2011-2012 estimated 24hr-2mo dairy calf mortality at 4.5% (Johnson *et al.*, 2017), similar to the current studies' finding of a 4.87% mortality rate between 0-2 months. Mortality rates have been reported to be highly variable between herds (Brickell *et al.*, 2009), with a recent study reporting a 2-56d mortality rate of 12.7% for one dairy herd (Mahendran *et al.*, 2017). Mortality rates in GB do not appear to have altered dramatically over time, with historic mortality rates up to six

months, estimated at around 5.2% in 1952 (Withers, 1952), extremely similar to the current study reporting 5.5%.

Previous research suggests a significant portion of on farm mortality occurs within early life, with the majority of calves dying within the first month (Gates, 2013; Santman-Berends *et al.*, 2014), and around two thirds of cattle mortality being within the first 4 months (Struchen *et al.*, 2015). There are a number of factors that affect calf mortality rates up to 3 months of age (Windeyer *et al.*, 2014), which are largely beyond the scope of this article discussion, however neonatal diarrhea and pneumonia are likely to play a significant role (Compton *et al.*, 2017).

Whilst calf mortality is a commonly reported metric in many countries, there are a wide variety of metrics being reported, and recent research has evaluated and assessed 10 definitions for calf mortality (Santman-Berends *et al*., 2019). Age classes for mortality studies also differ considerably between studies (Raboisson *et al.*, 2013), making comparisons challenging. A systematic review and meta-analysis, however, found the rate of perinatal (defined as 0-2d of age) mortality ranged from 3-9%, and was increasing over time, with neonatal (defined as 1d-weaning) mortality ranging from 5-11%, which was not found to have changed over time (Compton *et al.*, 2017).

As previously reported, there are several limitations to using BCMS databases for research purposes (Gates, 2013), particularly the potential exclusion of stillborn calves and early mortality prior to tagging. Previous estimates suggest around 7.9% of dairy calves die before 24 hours of age (Brickell *et al.*, 2009), and therefore the current studies estimates of mortality are likely to exclude perinatal mortality. Despite the legal requirement to register all deaths, including those before the registration and tagging of calves, there is a small risk that not all deaths/euthanasias that occur prior to tagging and registration are recorded. The absence of individual farm and calf level information available for this study meant some assumptions had to be made in order to calculate mortality figures, particularly that births and deaths were evenly distributed throughout months. Whilst this is important to recognize, the effects of these potential errors are likely to be small due to the large scale of the data and are unlikely to have significant bearing on the interpretation of the data. Data quality of CTS data has been reported to have improved over time (Green and Kao, 2007) and whilst it is unlikely to be completely free of error (Gates, 2014), the large scale of data being collected means extremely useful insights can be made.

2.5. Conclusions

The GB national cattle register provides an important resource in identifying neonatal mortality trends in the GB herd and will provide invaluable insights to the cattle industry if reported on a regular basis. Environmental conditions appear to play a significant role in calf mortality rates, and further research is needed to explore precise environmental factors likely to reduce calf mortality rates in GB.

To make the findings of this research accessible to farmers and practitioners, a "Mortality forecast" tool has been added to the University of Nottingham Herd Health Toolkit, where an estimate of national calf mortality can be predicted for a specific breed type and sex of calf, for a given environmental temperature and month of birth.

Chapter 3 Factors associated with daily weight gain in preweaned calves on dairy farms

This chapter discusses potential factors associated with growth rates in preweaned calves. A close derivative of this chapter has been published in Preventive Veterinary Medicine (Hyde *et al.*, 2021).

3.1. Introduction

The preweaning period is critical to the development and future performance of both dairy and dairy-cross beef calves on Great British (GB) dairy farms. A key outcome routinely measured across the preweaning period is average daily gain (ADG), which has been shown to be a crucial factor in the efficient rearing of productive dairy heifers (Chester-Jones *et al.*, 2017). Studies in GB have highlighted the importance of achieving a calving age of 23-25 months in minimizing rearing costs for dairy heifers (Boulton, Rushton and Wathes, 2017), and have suggested that increased bodyweight at 30d was associated with a reduced age at first calving (Brickell *et al.*, 2009). Alongside improvements in efficiency of heifer rearing, preweaning ADG has also been shown to significantly impact first lactation milk yield, with each 0.1kg of ADG being associated with a 85-113kg increase in milk yield during first lactation (Soberon *et al.*, 2012). Although this finding has not always been replicated in similar studies (Davis Rincker *et al.*, 2011; Kiezebrink *et al.*, 2015) a meta-analysis concluded that whilst other aspects of management are potentially more important than preweaning ADG, an increase in ADG above 0.5kg/d can enhance first lactation performance (Gelsinger, Heinrichs and Jones, 2016). Preweaning ADG is not only essential for the efficient rearing of productive dairy heifers, but also for efficient beef production (Greenwood and Cafe, 2007), which is important given the UK is currently the third largest producer of beef in Europe (DEFRA, 2018), and over 50% of this comes from dairy cross beef calves (AHDB, 2017). Although in one UK study only 28% of dairy farms routinely record heifer growth rates (Boulton, Rushton and Wathes, 2015), significant variations between farms have been reported (Bazeley *et al.*, 2016). Concerns have been raised over the environmental impact of both dairy and beef production, finding a high degree of heterogeneity in the environmental impact between producers (Poore and Nemecek, 2018), suggesting efficiency at farm level may play an important role in greenhouse gas emissions as well as productivity.

A variety of factors have been reported to affect ADG including colostrum supplementation, milk feeding protocol and composition (Brickell et al., 2009), total milk solids being fed, housing management (Johnson *et al.*, 2018) and environmental temperature (Shivley *et al.*,

2018). Whilst there is often an economic cost associated with alterations in preweaning calf management, the improved efficiency of rearing, reduced calving to conception interval and increased first lactation yields associated with increased ADG produce an economic saving to off-set higher preweaning costs (Boulton, Rushton and Wathes, 2017; Johnson *et al.*, 2018).

Whilst there are many potential factors influencing the ADG of preweaned calves, their relative importance remains unknown, and this is essential for optimal on-farm management decisions (Lima *et al.*, 2020). As discussed in Chapter 1, datasets containing large numbers of explanatory variables are more likely to contain potentially spurious correlations, which can confound conventional modelling approaches, resulting in inflated coefficients and over fitting, particularly when the number of predictors (p) exceeds that of the observations (n) (Kuhn and Johnson, 2013; Hastie, Tibshirani and Wainwright, 2015). One solution is the use of regularized regression (Zou and Hastie, 2005), where model coefficients are penalized unless there is a proportional improvement in model performance (Kuhn and Johnson, 2013). This penalization can take the form of reducing coefficients to near zero in the case of ridge regression (Hoerl and Kennard, 2006), or reducing the coefficient to zero in the case of lasso regression (Tibshirani, 1996). The elastic net algorithm makes use of both lasso and ridge regression, often outperforming both, whilst also producing a sparse model that can accommodate strongly correlated predictors (Zou and Hastie, 2005) often found when exploring farm management practices. Bootstrapping of regression models allows a robust estimate of coefficients (Breiman, 1996), and stability selection (Meinshausen and Bühlmann, 2010) can provide an estimate of variable stability; an estimate of the likely reproducibility of a variable effect in the target population. Used in conjunction with regularized regression, this approach has been utilized in animal health as a method of identifying a relatively small set of variables with a large and consistent influence on key outcomes (Lima *et al.*, 2020).

There are a wide variety of feeding, management and housing practices involved in achieving improved ADG rates in preweaned calves and whilst there have been several studies examining individual areas of calf feeding, environment and management, research examining these factors in a holistic manner is currently lacking. The aim of this study was to evaluate a wide variety of management variables using stability selection and regularized regression to identify factors most likely to affect preweaning ADG in calves on GB dairy farms.

3.2. Materials and methods

One hundred and twenty dairy farms were selected at random from a list of suppliers to a large GB supermarket. Selected farms were sent an initial information letter, followed up with a telephone call to recruit farms until 60 were recruited. One hundred and seven farms were contacted, with 47 declining to participate, resulting in 60 participating farms. Recruited farms were visited between 17th December 2018 and 14th February 2019 at which time a detailed calf management questionnaire was conducted, alongside the measurement of calf housing dimensions and environmental parameters.

3.2.1. Management questionnaire

The management questionnaire contained 374 questions, including sections on general farm background, farmer demographics, calving area management, current calf morbidity and mortality, colostrum management, milk feeding, weaning and building management. A full copy of the questionnaire is available in *Appendix i*

3.2.2. Building measurements

Building parameters were recorded for all buildings in which the calves spent ≥24hr including calving pens. The mean number of cows or calves typically within the pen and building were recorded as a farmer estimate, as was the number of pens within the building and the number of days spent in each environment. The shape of the pens and building were recorded as was the roof design (i.e., dual-pitched, mono-pitched, hutch etc) and ridge outlet design (open, cranked, covered etc).

Bedding depth, roof coverage (% of housed area covered by a roof) and the number of mechanical ventilation fans present was recorded numerically. The availability of water, sharing of water both within and between pens and the presence of water over bedding was recorded. The presence of cobwebs, bedding wetness/contamination, and ammonia levels were scored subjectively on a 0-10 scale (10 being "high") and nesting score on a 1-3 scale (1: calves' legs fully visible in recumbency, 2: calves' legs partially visible in recumbency, 3: calves' legs not visible in recumbency) (Lago *et al.*, 2006).

Building dimensions were recorded using a laser measurer (LDM-100, CEM, Kolkata) including the widths and lengths of all pens and buildings, the height to eaves and ridges of buildings, with details being collected on wall material (concrete, space boarding, mesh etc), proportion of wall being solid/ventilated, and measurements of bedded/non-bedded areas. The airspace per pen and airspace per shed was calculated from recorded dimensions, as was typical stocking density (m^2 available per animal calculated using farmer estimated mean

stocking numbers). Building dimensions were calculated both as individual measurements for each feeding period, and as mean values amalgamated across the entire preweaning period (e.g. a calf being housed for 7d in a building whilst being fed colostrum with a ridge height of 2m followed by being housed in a building with a ridge height of 4m for 63d whilst being fed milk replacer (MR), would have a feed1 ridge height of 2m, a feed2 ridge height of 4m and a mean ridge height of 3.8m).

Measurements of environmental conditions were taken on the day of visit including minimum/maximum airspeed at both human (1.5m) and calf (0.5m) level, environmental inside/outside temperature, relative humidity, minimum/maximum light intensity (lux) using a handheld monitor (Enviro-meter, Fisher scientific, Leicestershire), as well as temperature of all four walls, bedding and outside ground temperature via a laser thermometer (IR-801, ATP, Leicestershire).

Data loggers (EL-CC-2, Lascar, Wiltshire) were installed in all separate calf buildings on each farm at 1.5m height, up to a maximum of 3 buildings, depending on the number of buildings used. A data logger was installed outside the main calf shed at 1.5m height on each farm. Loggers were set to record temperature and humidity every 10 minutes and were posted back to the authors via a prepaid envelope in July 2019.

3.2.3. Feeding measurements

On farms that were feeding milk replacer (MR), the youngstock manager was asked to measure the quantity of MR powder as typically prepared for feeding, which was then weighed using digital weigh scales (CL series, OHAUS, Nänikon) so the true weight could be recorded in addition to the desired weight. Details of the MR ingredients and constituents were also recorded. Water temperature used to mix MR powder was measured using a laser thermometer (IR-801, ATP, Leicestershire). Farms not feeding milk replacer (and thus constituents data could not be collected) were recorded as “NA” for milk replacer constituent variables.

3.2.4. Colostrum and immune transfer

Farmers were trained in refractometer use during the farm visit, and a Brix refractometer (Model RHB-325G with 0-32% range, YHequipment, Shenzhen) was provided to all participating farmers. Farmers were instructed to record the Brix % of colostrum samples as least weekly. Colostrum bacteriology sampling kits were provided to farmers, consisting of six sample pots containing glycerol with instructions to sample the next 6 colostrum samples available (within one month) at the point of calf feeding (i.e. from the teat/bottle/tube depending on colostrum feeding method) which were frozen immediately on-farm as

described in previous colostrum studies (Mcaloon *et al.*, 2016), and asked to post the samples within one month of the first sample being taken. Ice packs were provided for samples which were sent directly to the laboratory where temperature checks were performed on arrival. Total bacterial counts were performed after 72hr incubation at 30°C and coliform counts were performed after 72hr incubation at 37°C.

Farmers were asked to consider blood sampling a sample of calves for passive transfer of immunity with their routine veterinarian, but this was voluntary and to be performed only if there was deemed to be a valid clinical justification by the local veterinarian.

3.2.5. Farmer recording

Calf weight at birth and weaning were collected by farmers using a weigh tape which was provided to all farmers (MSD animal health, Milton Keynes, UK). All farmers were trained in the correct use of the weigh tapes, and were also asked to record the breed, sex, time period between birth and colostrum feeding and whether calving assistance was required. Farmers were contacted every 2-4 weeks via text, email or telephone throughout the trial to encourage compliance.

3.2.6. Descriptive analysis

All data analysis was conducted in R (R Core Team, 2020). Birth and weaning weights and dates were used to calculate an average ADG for each calf. Calf level variables were converted to farm level variables, i.e., percentage births receiving calving assistance, mean time from birth to colostrum feeding. An ADG dataset was created by filtering any calves without either a birth or weaning weight and calculating a mean ADG at farm level. Morbidity and mortality datasets were created by filtering farms that did not record any disease or death recording within the trial period.

Blood sample and colostrum brix results were used to calculate a percentage failure of passive transfer and low colostrum quality rate respectively for each farm. “Failure” of passive transfer was defined as <5.2g/dl total protein, <7.8% Brix or <14.6 ZST (Zakian *et al.*, 2018), and colostrum was determined as being a “failure” in terms of quality when <22% Brix (Quigley *et al.*, 2013). Similarly, the percentage of colostrum samples failing in terms of total bacteria count (TBC) and coliform count (CC) was calculated for each farm, with a “failure” being when >=100,000 for TBC and >=10,000 for CC as previously suggested by McGuirk and Collins (2004).

The day of logger installation and the last 2 weeks of recording were removed to ensure temperature readings during installation or posting were not included in any subsequent analysis. Data from the loggers were analysed both individually and amalgamated into farm

level “inside” and “outside” mean, median, max and min measurements. Mean, median, min, max and SD of both temperature and humidity were calculated for all calves for the first 7, 30 and 60 days of life for inside building and outside building temperatures respectively.

3.2.7. Statistical analysis

Some variables where causality was deemed implausible were excluded, e.g., frequency of milk recording, farmer attitudes to building importance etc, and categorical variables were removed when <2 levels were present. All numeric variables were centred and scaled (divided by one standard deviation) using “center” and “scale” inside the preProcess function within the caret package (Kuhn. *et al.*, 2018). Farm management variables with missing data were converted to categorical variables including an “Unknown” category for missing data. Missing data for the percentage of colostrum samples failing on CC or TBC count (n=1) and temperature/humidity inside (n=3) and outside (n=5) buildings were imputed using the rfImpute function from the randomforest package (Liaw, 2018).

Elastic net models employ a balance of lasso ($\ell 1$) and ridge ($\ell 2$) penalties and in the current study took the form

$$\lambda \sum_{j=1}^{p} \left(\alpha \beta_j^2 + (1-\alpha)\left|\beta_j\right|\right),$$

where the elastic net penalty is represented as the summation of a balance of lasso and ridge penalties for *j* of *p* predictors (Zou and Hastie, 2005). $|\beta|$ represented absolute values of the regression coefficients. Hyperparameters that represent the penalty (λ) and the relative proportion of penalisation on either the sum of the square of the coefficients or the unsquared coefficients (α) were optimised by 10 x 5-fold cross validation to minimise MAE.

A preliminary elastic net model was created using the *glmnet* (Friedman, Hastie and Tibshirani, 2010) and *caret* (Kuhn. *et al.*, 2018) packages to inform grid parameters for α and λ. A full elastic net model was then created, again using *glmnet* and *caret*.

Based on the α and λ values in of the full model, a dense and sufficiently wide grid of α and λ values were created for future bootstrapped models. One thousand bootstrap samples were taken to enable estimations of bootstrap intervals (Efron and Tibshirani, 1994), and an elastic net model was built for each sample using the *glmnet* package (Friedman, Hastie and Tibshirani, 2010). Five-fold cross validation was repeated 10 times for each model. The dense grid of α and λ values was used for tuning, and optimal models were selected for each bootstrap sample based on minimising the mean absolute error (MAE). Final model coefficient values for all variables were saved for each iteration of the bootstrap sample process, and the percentage of models which contained each variable was subsequently

calculated as a measure of variable stability. Coefficients were converted back to original units by dividing the coefficient by the standard deviation (SD) used to pre-process the variable from the original dataset. Models were interpreted utilising both stability and coefficient distributions as follows. A 90% bootstrap confidence interval (BCI) was calculated for each variable and a bootstrap p-value defined as one minus the proportion of coefficient estimates on the majority side of zero (proportion below zero if the mean coefficient was above zero, and proportion above zero if mean coefficient was above zero). Variables with a stability >50% and a bootstrap p-value <0.025 were deemed to be both stable and have an effect size with a high probability of being > or < 0 and were therefore selected to comprise a final model.

3.3. Results

3.3.1. Descriptive statistics

Farms were predominantly in the Southwest and Midlands of England with 46, 11 and 3 in England, Scotland and Wales respectively. Herd size was a mean of 312, ranging from 72 to 2100 milking cows, with a mean milk yield of 9323 L/305d, ranging from 6500-12000 L/305d. Fifty farms milked cows using a conventional milking parlour, with 10 being robotically milked.

A total of 6,973 weights of 4,552 unique calves were recorded from 37 farms, with twenty-three farms not returning any calf weight data despite repeated contacts. Seven of the 37 farms had poor quality data which meant it was not possible to calculate ADG and were excluded, resulting in 30 farms in the final dataset. Four of these 30 farms did not record date of weaning, and these were estimated using mean age of weaning as recorded during the farm management questionnaire. As a cross-check, models were repeated without these four farms included to ensure no significant alterations in model outcomes. Individual calves from the final 30 farms with weights at selling rather than weaning were removed (n=318), and subsequently calves without a birth or weaning date (n=55) were excluded from the analysis.

After processing and filtering, the final dataset of calf weights included 1,014 calves that had both a birth and weaning weight from 30 farms. The mean ADG for each farm was calculated as an outcome variable at farm level. Six hundred and eighty-nine colostrum brix recordings, 249 colostrum bacteriology and 280 calf blood sample results were returned from 26, 39 and 18 farms respectively. One hundred and three data loggers were returned from 40 farms which resulted in 2,459,108 recordings for temperature and humidity recordings respectively. Fewer farms returned data for morbidity and mortality, and the final

datasets for diarrhoea included 2,586 calves from 26 farms. The final dataset for pneumonia included 2,071 calves from 15 farms, and the final dataset for mortality included 2,013 calves from 15 farms.

Of the 30 farms included in the final dataset, including data from 1,014 calves, the mean ADG was 0.79kg/d (range 0.49-1.06kg/d, SD 0.13). For morbidity and mortality datasets, the mean incidence risk of calves experiencing scour was 21.96 % (range 0.32-59.09%, SD 16.65), with mean incidence risk for pneumonia at 11.24 % (range 1.76-35.48%, SD 11.38) and mean incidence risk for mortality at 4.23 % (range 0.75-11.54%, SD 3.01). Calves were fed a mean of 5.62L/d of either milk or milk replacer during the preweaning period (2.93-9.24L/d, SD 1.37) and were stocked at a mean rate of 2.59m^2/calf (1.14-5.00m^2/calf, SD 0.93). Mean temperatures within the first month of life were very similar between both inside and outside shed measurements, with a mean temperature of 10.35 C (6.36-14.90, SD 1.71) and 9.85 (6.46-13.28, SD 1.68) for inside and outside shed temperatures respectively. Calving pens were cleaned out a mean of every 32d (3.5-105d, SD 27.67) and group calf pens were cleaned out a mean of every 55d (5-180d, SD 38.22). The maximum period calves would go without colostrum had a mean of 8.8hr (2-24hr, SD 4.3) and the mean proportion of colostrum samples failing in terms of TBC and CC was 34.9% (0-100%, SD 32.4) and 10.7% (0-83.3%, SD 22.6) respectively.

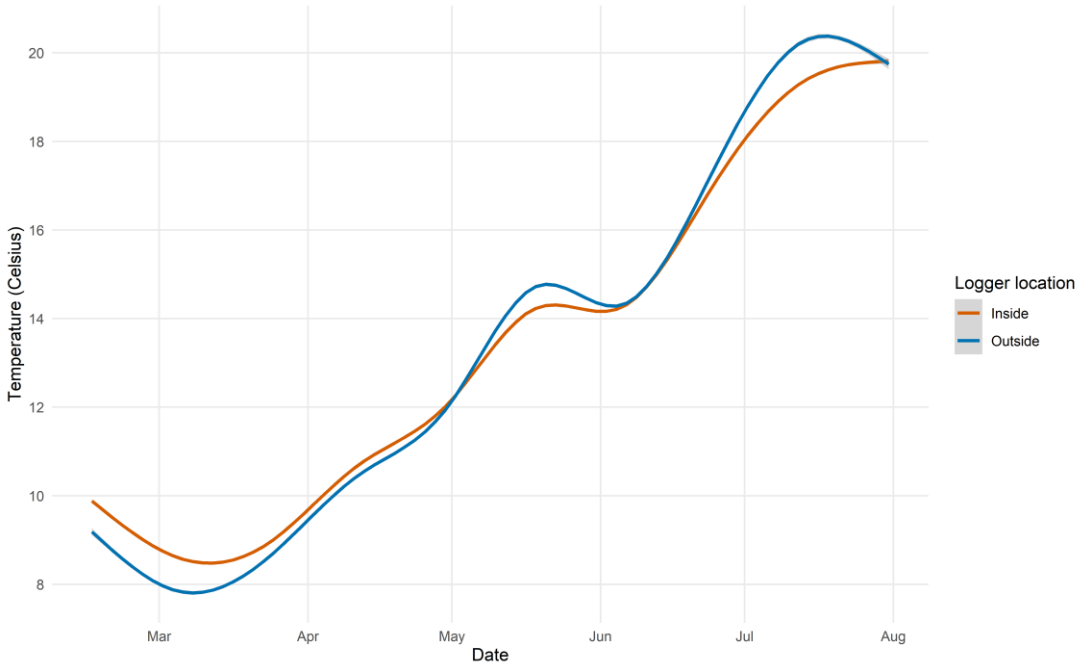


Figure 3-1: Mean daily temperature (Celsius) by date for data loggers placed inside and outside calf buildings.

Temperature and humidity analysis illustrated minimal differences in mean daily temperature between inside and outside temperatures (Figure 3-1). Hourly temperature

analysis demonstrated that calf buildings were generally slightly warmer overnight than the outside temperature and were slightly colder during the day (Figure 3-2), however these differences were relatively small, generally in the region of 2°C.

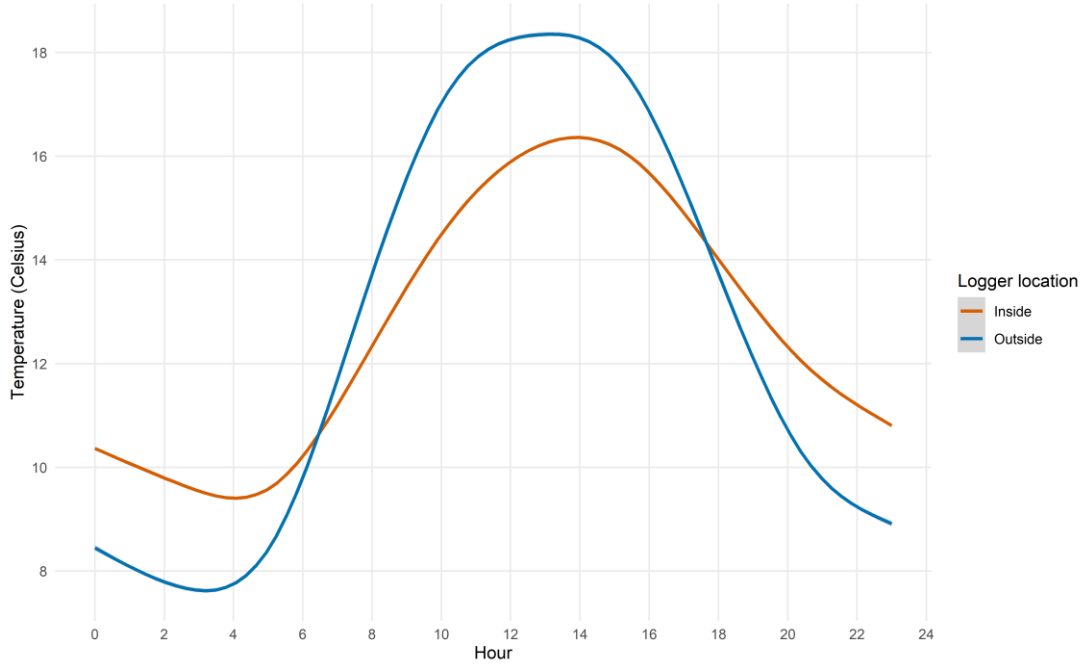


Figure 3-2: Mean hourly temperature (Celsius) for data loggers placed inside and outside calf buildings.

3.3.2. Factors associated with growth rates

Two hundred and ninety-three variables were available for predicting ADG at farm level which would be included in the final analysis. Preliminary elastic net models indicated optimal α and λ values at 0.55 and 0.039 respectively. A dense grid including α values of 0, 0.2, 0.4, 0.6, 0.8, 1 and λ values of 0.001, 0.002, 0.003, 0.004, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.1 was used for bootstrapping. A median of 0.4 and 0.001 was selected throughout the 1,000 bootstrap sample repeats for α and λ respectively.

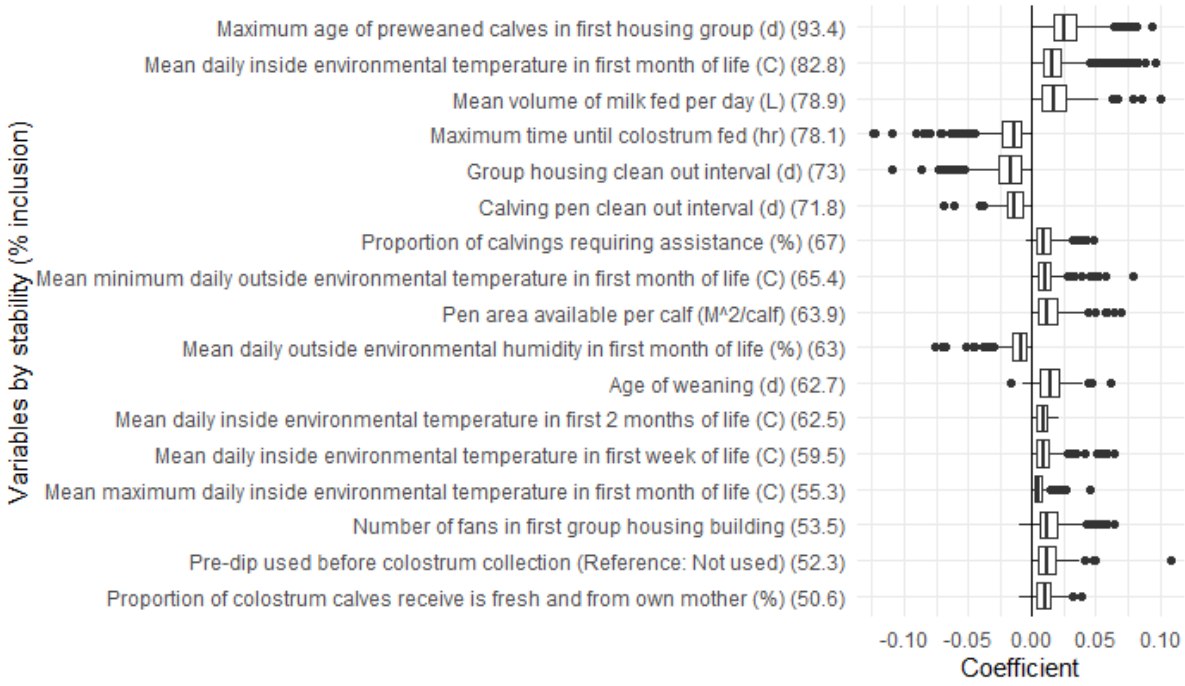


Figure 3-3: Coefficient distributions and variable stability for the outcome ADG for variables selected in at least 50% of models across 1000 bootstrapped samples. Coefficient estimates for numeric variables represent the change in average daily gain (kg/d) associated with a change of 1SD in each variable

Of the 293 available variables, 18 variables were selected in at least 50% of the models as shown in Table 3-1. Variables with a stability >50% and bootstrap p-value <0.025 were included in the final model, resulting in 17 variables being selected. Model coefficients, illustrated in Figure 3-3 are depicted as the effect of a 1SD change in each independent variable on ADG (kg/d). The stability and bootstrap p-value of all variables with stability >50% are illustrated in Figure 3-4.

An older maximum age within first preweaned housing group (the maximum age a calf might reach in the first housing group) was associated with increased ADG, with a stability of 93.4%, and a coefficient of 0.001 (90% BCI: 0.000-0.002kg/d) indicating a mean increase of 10g/d for every 10d age for the oldest calf within the first preweaned group. There was also an effect of increased mean volume of milk/MR fed on ADG, with a stability of 78.9% and a coefficient of 0.012 (90% BCI: 0.001-0.024kg/d), indicating an increase in feed volume of 1L was associated with an increase in ADG of around 12g/d. An increased mean daily inside environmental temperature within the first week, month or two months of life was also associated with improved ADG, with stabilities of 59.5%, 82.8% and 62.5% respectively, with temperature in the first month of life (°C) having a coefficient of 0.012 (90% BCI: 0.002-0.037kg/d), suggesting a 5°C increase in environmental temperature would increase ADG by 60g/d. Increases in the maximum time to colostrum feeding was associated

with a decrease ADG, with a stability of 78.1% and coefficient of -0.004 (90% BCI: -0.010 - 0.000kg/d), suggesting a 6hr delay in max colostrum feeding time would decrease ADG by 24g/d over the preweaning period.

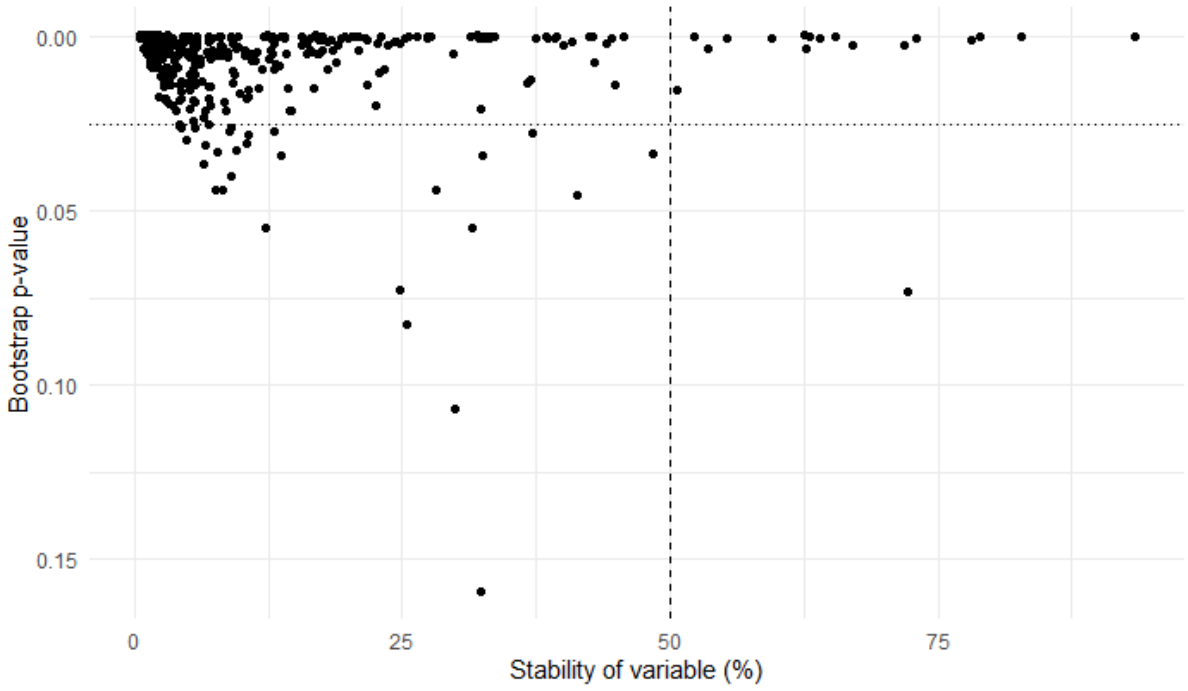


Figure 3-4: Bootstrap p-value by stability of variables (%). Points jittered to avoid overlapping. Variables selected for final model were above 50% stability (dashed line) with a bootstrap p-value of <0.025 (dotted line).

Longer periods between the cleaning out of group housing and calving pens were associated with decreased ADG, with 73.0% and 71.8% stability respectively, with coefficients of -0.001 (90% BCI -0.002 – 0.000kg/d) and -0.001 (90% BCI -0.001 – 0.000kg/d), suggesting that a 1wk delay in cleaning out of either the calving pen or group housing would be associated with a 7g/d reduction in ADG, and a simultaneous 1wk delay in both calving pen and group housing would be associated with a 14g/d reduction. Increased space allowance per calf was associated with an increased ADG, with a stability of 63.9% and coefficient of 0.010 (90% BCI 0.001-0.025kg/d), indicating an increase in ADG of 10g/d for each additional m^2 of space per calf. Model coefficients for morbidity and mortality outcomes are presented in Figure 3-5.

	Stability (%)	Mean coefficient	90% Bootstrap confidence interval	Bootstrap P-value
Maximum age of preweaned calves in first housing group (d)	93.4	0.001	(0.000 - 0.002)	<0.001
Mean daily inside environmental temperature in first month of life (C)	82.8	0.012	(0.002 - 0.037)	<0.001
Mean volume of milk fed per day (L)	78.9	0.012	(0.001 - 0.024)	<0.001
Maximum time until colostrum fed (hr)	78.1	-0.004	(-0.01 - 0.000)	0.001
Group housing clean out interval (d)	73	-0.001	(-0.002 - 0.000)	<0.001
Proportion of colostrum calves receive is colostrum replacer (%)	72.2	-0.001	(-0.003 - 0.000)	0.073
Calving pen clean out interval (d)	71.8	-0.001	(-0.001 - 0.000)	<0.001
Proportion of calvings requiring assistance (%)	67	0.001	(0.000 - 0.003)	0.002
Mean minimum daily outside environmental temperature in first month of life (C)	65.4	0.009	(0.001 - 0.020)	<0.001
Pen area available per calf (M2/calf)	63.9	0.010	(0.001 - 0.025)	<0.001
Mean daily outside environmental humidity in first month of life (%)	63	-0.004	(-0.010 - 0.000)	<0.001
Age of weaning (d)	62.7	0.002	(0.000 - 0.004)	0.004
Mean daily inside environmental temperature in first 2 months of life (C)	62.5	0.005	(0.001 - 0.009)	<0.001
Mean daily inside environmental temperature in first week of life (C)	59.5	0.007	(0.000 - 0.019)	<0.001
Mean maximum daily inside environmental temperature in first month of life (C)	55.3	0.001	(0.000 - 0.003)	<0.001
Number of fans in first group housing building (n)	53.5	0.025	(0.002 - 0.062)	0.004
Pre-dip used before colostrum collection (Reference: Not used)	52.3	0.012	(0.001 - 0.030)	<0.001
Proportion of colostrum calves receive is fresh and from own mother (%)	50.6	0.000	(0.000 - 0.001)	0.015

Table 3-1: Variable stability, coefficient estimates, 90% bootstrap confidence intervals and bootstrap p-value for variables present in the final model of calf preweaning average daily gain (kg/d). Coefficient estimates for numerical variables relate to the effect of a 1-unit change in average daily gain.

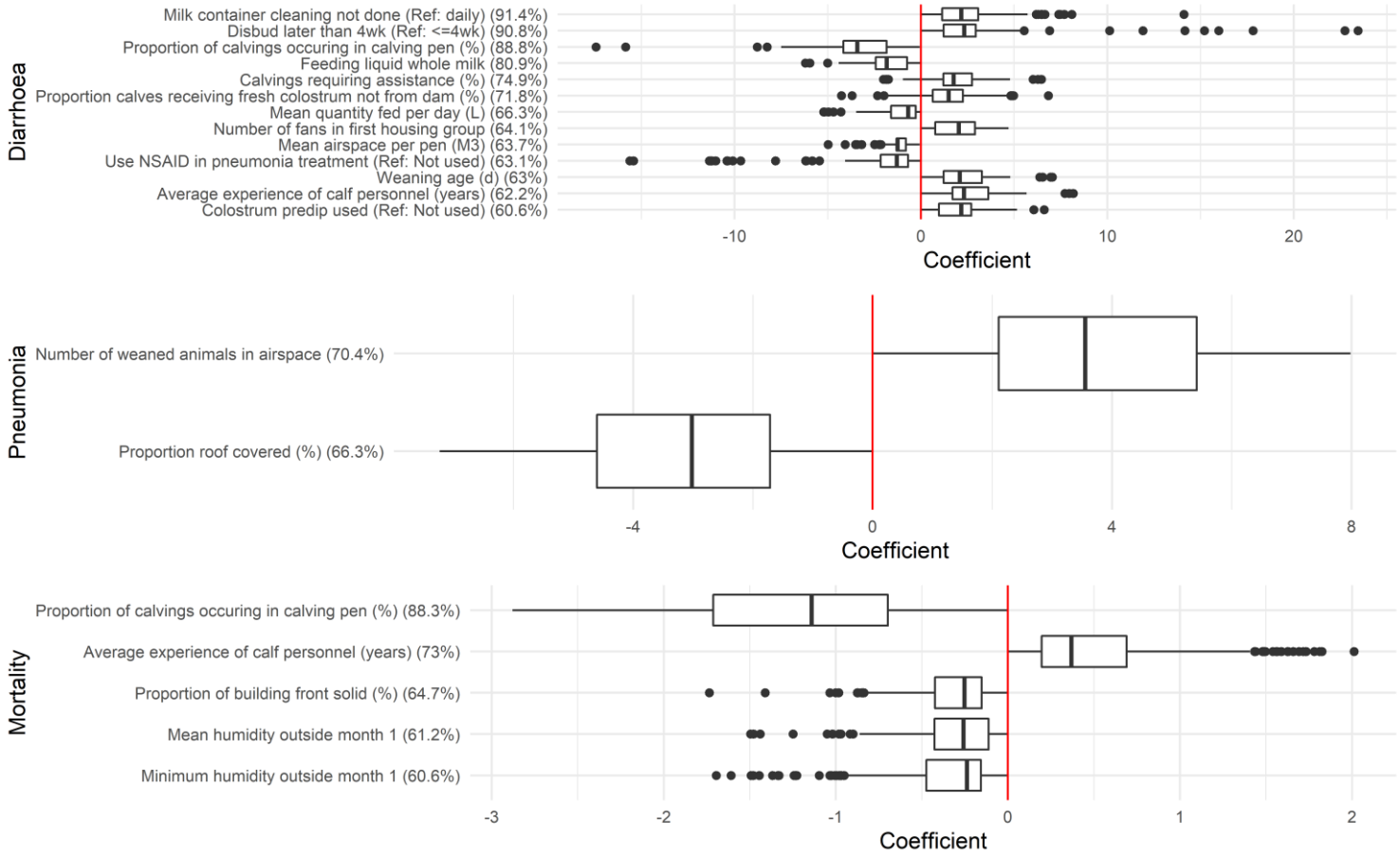


Figure 3-5: Coefficient distributions and variable stability for the morbidity and mortality outcomes for variables selected in at least 60% of models across 1000 bootstrapped samples. Coefficient estimates for numeric variables represent the change in morbidity or mortality rate (%) associated with a change of 1SD in each variable.

3.4. Discussion

Of the 293 variables available, 17 were found to have both high stability and an effect size with a low bootstrap p-value when predicting mean farm ADG. Many of these variables represent similar practical aspects of management and largely focus on stocking demographics, milk/colostrum feeding, environmental hygiene and environmental temperature.

Increases in several environmental temperature variables at a variety of age ranges appeared to be associated with improved ADG. The lower critical temperature is thought to be around 20°C for calves up to 3 weeks old (National Research Council, 2001) however the mean UK temperature in 2019 was only 9.4°C, with winter 2018/19 temperatures averaging only 5.2°C (Met Office, 2019). As neonatal calves are likely to be far below their lower critical temperature for much of the year in GB, it is perhaps unsurprising that lower environmental temperatures were associated with reductions in ADG in the current study, as calorific requirements are likely to increase in cold temperatures (National Research Council, 2001). Calves in GB are generally housed in naturally ventilated buildings, and temperatures inside similar calf buildings have previously been found to be similar to outside environmental temperatures across Europe (Seedorf *et al.*, 1998). These findings were replicated in the current study, with mean daily temperatures recorded inside calf buildings being almost identical to those outside on each farm as shown in Figure 3-1. Since the design of the building appears to have minimal effect on environmental temperatures, it may be that active heating elements (such as heat lamps) could have a positive effect on calf ADG. Methods to limit weather exposure and therefore reduce heat loss through convection should also be further explored. Negative effects of colder environmental temperatures on calf mortality at national level have recently been reported in GB as described in Chapter 2, and the effects of interventions that improvements in the calves' thermal environment on both mortality and ADG could be directly explored in future studies. It is worth noting that the positive effect of increasing temperature on ADG is likely to be country-specific, and caution should be taken extrapolating these results to countries with differing weather conditions to GB. Despite the apparent importance of environmental temperature, calf jacket usage did not have a consistent beneficial effect on ADG, similar to previous findings (Earley *et al.*, 2004; Scoley, Gordon and Morrison, 2019).

Increased milk volume was found to improve ADG during the preweaning period. Similar results have been found in a previous studies using a meta-analytic approach (Hu *et al.*, 2020), and whilst concerns have been raised that feeding calves a higher allowance of milk increased total feed costs, research has demonstrated a decreased total cost per kg of

liveweight gain with increased milk feeding (Hawkins *et al.*, 2019). Despite research suggesting calves will voluntarily drink up to 13.3L (95% CI 12.4-14.2L) of milk by 26d of age (Curtis *et al.*, 2018), recent UK surveys suggest calves are routinely being fed 4-8L, with considerable variation in feeding practices due to a lack of scientific consensus in effective protocols to promote growth and future performance (Palczynski *et al.*, 2020). Calves fed only 6L a day have been reported to show chronic signs of hunger (Rosenberger *et al.*, 2017), however, only 8 out of the 30 farms in the final dataset reported feeding greater than 6L/calf on average. Whilst there are likely to be objective gains in terms of productivity when increasing milk feeding, the benefits to calf welfare of increased milk volume allowance should also be considered.

The association of an increased maximum age of preweaned calves in the first housing group (the maximum age an animal might reach whilst housed in their first group) with increased ADG suggests that calves remaining within one housing group throughout weaning may benefit from higher ADG than those being housed in several housing groups within the preweaning period. This may be in part due to calves' tendency to learn from their companions, potentially increasing early intakes. There may also be an effect of minimising group changes in preweaned calves, as calves housed for longer within the first housing group would consequently experience fewer social group changes in early life than those spending a shorter time in the first housing group. A review into the effects of group housing found that social housing improves intakes and weight gains (Costa, von Keyserlingk and Weary, 2016), which has been reinforced by more recent studies reporting increased growth rates when group housed rather than housed individually (Curtis *et al.*, 2018; Johnson, Nair and Wathes, 2019). Whilst there are concerns that group housing might be disadvantageous from an disease transmission perspective, any health risks associated with group housing can likely be mitigated with appropriate management (Costa, von Keyserlingk and Weary, 2016).

An increased interval between the complete cleaning out of both group housing and calving pens was consistently associated with a reduced ADG. Previous research has suggested that neonatal calf diarrhea is negatively associated with ADG (Windeyer *et al.*, 2014). These results may support findings from the current study that environmental hygiene of both the calving pen and group housing environment are consistently important for improving ADG, potentially through reduced disease levels.

A longer maximum time until colostrum feeding, a decreased percentage of calves receiving fresh colostrum from their own mother and failure to use a pre-dip prior to colostrum collection were all consistently associated with reduced ADG. This suggests that colostrum

feeding timing, substance and hygiene during collection may all play an important role in ADG. Failure of passive transfer has previously been negatively associated with ADG (Windeyer *et al.*, 2014). Timing, substance and importance of maintaining low bacterial levels within colostrum in reducing the risk of failure of passive immunity transfer (FPT) has been reported in previous studies (Godden, 2008; Gelsinger, Jones and Heinrichs, 2015). As FPT is known to increase the risk of disease (Raboisson, Trillat and Cahuzac, 2016), the effects of colostrum management on ADG may be indirect effects due to reduced rates of FPT reducing the incidence of neonatal calf disease and therefore mitigating the negative effects of disease on ADG (Windeyer *et al.*, 2014). This was challenging to investigate in the current study, as a low number of FPT data was returned from farms, with 12/30 farms having unknown levels of FPT.

An increased frequency of veterinary visits was also associated with a higher ADG. The stability of this variable however was only 43.9% with a mean coefficient only -0.001 kg/d per additional 1d between veterinary visits (90% BCI -0.003 – 0.000, bootstrap p-value 0.045), suggesting that other variables may initially result in a larger effect size on a greater number of farms than an increased rate of veterinary visits. Nonetheless, this result suggests veterinarians can play an important role in the health and productivity of preweaned calves regardless of specific calf health training. Similar findings have been reported in studies examining factors associated with mortality, suggesting that the absence of active/routine calf health involvement from a veterinarian was associated with a higher rate of calf mortality (Renaud *et al.*, 2018).

There were occasionally variables that despite having low stability still appeared to have a large effect size such as increasing the age (d) at which dry feed is provided, with a coefficient of -0.003 (90% BCI -0.007-0.000) and bootstrap p-value of 0.002 despite a relatively low stability of 40.0%. Similarly increasing the age (d) at which free water access is provided had a coefficient of -0.003 (90% BCI -0.008-0.000) and bootstrap p-value of 0.013. This suggests that whilst these variables are not necessarily important on the majority of farms, there are certain situations where they might have a larger effect size than other variables, with a 7d delay in either the provision of dry food or free water access after birth being associated with a 21g/d decrease in ADG across the preweaning period, and a simultaneous delay in both dry food and free water access being associated with a 42g/d decrease in ADG.

There were numerous calf management factors identified in this study with the potential for major improvements (relatively large effect size) to ADG on the majority of farms (high stability). Conversely there were some factors that might have a minor impact (relatively

small effect size) on the majority of farms (high stability). It would be impractical however for veterinary advisors to suggest implementation of a large number of management interventions aimed at improving ADG, when many of these would have limited impact. The identification of key factors likely to have the largest impact on the ADG (effect size) of preweaned calves on the highest proportion of farms (stability) is consequently extremely important, enabling veterinary advisors to suggest the small number of interventions likely to have maximum gain in the majority of cases. Whilst the methods utilised in this research have identified several stable variables with relatively large effect sizes, it is possible a number of variables with an effect on ADG might have remained undetected due to sample size constraints. The use of the elastic net algorithm means a conventional sample size calculation is unlikely to be appropriate, however by taking the standard deviation from the current research (0.13kg/d) and 15 farms per group, conventional sample size calculations indicate an 80% chance of detecting a difference of 0.14kg/d in ADG, at a significance level of 0.05. Whilst this method of sample size calculation would not be appropriate when using elastic net, it suggests that variables with relatively small effect sizes might only be detected if a larger sample size was available.

A potential limitation to the estimation of ADG in this research was the use of weigh tapes rather than calibrated weigh scales. The use of weigh tapes has been shown to provide reliable estimates of bodyweight in young calves (Heinrichs, Rogers and Cooper, 1992), and although there have been concerns about the accuracy of tape measures (Dingwell *et al.*, 2006; Heinrichs *et al.*, 2007) further validation studies have suggested this method to be sufficiently accurate (Bond *et al.*, 2015), with the use of weigh tapes being suggested as practical when scales are unavailable or impractical (Atkinson, von Keyserlingk and Weary, 2017). It has also been recently demonstrated that the use of multiple calves for the estimation of ADG by weigh tape dramatically reduces the error rates in group ADG estimation (Sherwin *et al.*, 2021), further decreasing the chances that there was a significant degree of error introduced through the use of weigh tapes. The use of a birth and weaning weight measurements should ameliorate user variability to a degree as users consistently overestimating/underestimating birthweights would be likely to consistently overestimate/underestimate weaning weights, resulting in relatively accurate ADG estimates at farm level. There is the potential that farm management practices varied from responses given in the management questionnaire, however the authors feel that farmers were generally honest and accurate with their responses, even when these responses might not represent best practice. There may also be limitations in the collection of environmental temperature, as data loggers were placed at 1.5m height, and thus may not accurately represent the calves' local environment when in recumbency. Whilst logger placement at

calf level may be desirable, it is logistically more challenging due to the propensity of calves to vigorously explore novel items in their environment, potentially damaging the loggers. Preliminary trials suggested that differences between 0.5m and 1.5m height within calf housing had relatively minor differences in absolute temperature, and followed similar trends in temperature changes, without the risk of damage to the data loggers.

Fewer farms adequately recorded morbidity and mortality outcomes than ADG outcomes, and the failure of farmers to accurately record health data has been reported previously (Sivula *et al.*, 1996; Virtala *et al.*, 1996; Johnson *et al.*, 2017). Due to the relatively poorly recorded morbidity and mortality data, the subsequent potential for identifying factors associated with these outcomes was reduced. Although the identification of risk factors was more challenging for morbidity and mortality outcomes than ADG, there was a relatively high level of duplication in variables identified across morbidity, mortality and growth outcomes. It is likely that factors associated with reductions in morbidity and mortality might also be related to improved growth rates and vice versa. Whilst this it was not possible to test this hypothesis in the current study, the relationship between calf disease and growth rates has been demonstrated previously (Windeyer *et al.*, 2014). As a specific example, the feeding of higher levels of milk was identified as being associated with both increased ADG and reductions in diarrhoea. Reviews into cow-calf separation report positive effects of cow-calf contact in reducing diarrhoea cases, potentially due to the increased quantity of milk consumed by these animals (Beaver *et al.*, 2019), and it is likely that increased levels of milk being fed might have benefits both in terms of health and productivity. Irrespective of any links between factors associated with diarrhoea and ADG, it would seem worthwhile pursuing the improvement of farm management factors identified as being associated with multiple health and production outcomes.

The generalisability of this study is unknown since there may be some differences between farms randomly selected from a supermarket group and the general dairy farm population in GB. However, since farms were initially selected for inclusion within the supermarket group based on convenient milk tanker routes, any initial selection bias is likely to be limited. Participation in the study was voluntary which may have introduced a degree of bias by selecting more proactive farmers, although the majority of farms contacted agreed to participate in the study (55% of 110 farms contacted), with a similar proportion of participating farms returned high quality data (50%). Whilst there is the potential for farms within the final dataset to vary from the general population, we believe they are likely to be a largely representative sample of GB dairy farms.

An important additional limitation of this research is that associations identified during the observational study should not be interpreted as causal, and further research investigating the effects of these interventions as a randomised controlled study is described in Chapter 5.

3.5. Conclusions

Increasing the maximum preweaned age within the first housing group, increasing both milk feeding volume and environmental temperature, ensuring regular cleaning out of both group housing pens and calving pens and ensuring rapid feeding of colostrum were found to be particularly important in improving ADG at farm level.

ADG in the preweaning period has been shown to be extremely important in the efficient rearing of both dairy and dairy cross beef calves. There are numerous variables likely to affect ADG in preweaned calves, and it is essential that veterinary advisors are able to target a small number of stable variables likely to have the largest effect size. By performing elastic net regression in conjunction with stability selection, a small number of stable variables have been identified that are most likely to have the largest effect size on ADG in preweaned calves. Further research should be conducted to test the impact of interventions to improve these consistently important management factors on the ADG of preweaned calves on GB dairy farms.

Chapter 4 Quantitative analysis of colostrum bacteriology on British dairy farms

This chapter provides a benchmark for colostrum hygiene levels in Great Britain, and potential factors associated with high bacterial levels are discussed. A close derivative of this chapter has been published in Frontiers in Veterinary Science (Hyde *et al.*, 2020).

4.1. Introduction

Bovine neonates are born agammaglobulinemic (Barrington and Parish, 2001) and consequently must acquire immunity via the ingestion of appropriate quantities of high quality colostrum within the first few hours of life (Godden, Lombard and Woolums, 2019). A failure of passive immunity transfer (FPT) in dairy calves has been associated with an increased risk of preweaning morbidity and mortality, as well as longer term effects such as increased age at first calving, reduced first and second lactation milk production and an increased risk for culling within the first lactation (Robison, Stott and DeNise, 1988; Wells, Dargatz and Ott, 1996; Faber *et al.*, 2005; Godden, Lombard and Woolums, 2019). A recent meta-analysis concluded that calves experiencing FPT are 1.5 times more likely to be treated for diarrhoea, 1.8 times as likely to be treated for respiratory disease and 2 times as likely to die (Raboisson, Trillat and Cahuzac, 2016). Despite the clear negative implications, recent research suggests FPT is common in UK dairy calves, with around 26% experiencing FPT as measured by total protein (TP) < 5.6g/dl (MacFarlane *et al.*, 2015).

FPT has been associated with suboptimal colostrum feeding volume, timing, quality and also microbiological hygiene (Godden, Lombard and Woolums, 2019). Microbiological contamination of colostrum can not only represent a significant risk for FPT through reduced efficiency of immunoglobulin absorption (Gelsinger, Jones and Heinrichs, 2015), but also act as a vehicle for the transmission of pathogenic organisms to the neonatal calf (McAloon *et al.*, 2016). Higher bacterial levels have been hypothesised to reduce IgG absorption by the binding and neutralising of Ig by bacteria, pathogenic bacteria damaging intestinal epithelial cells and reducing permeability to Ig, and nonspecific pinocytosis of bacteria blocking the absorption of Ig molecules (Godden *et al.*, 2012). In addition to effects on FPT and disease early in life, colostrum hygiene has also been identified as a critical control point in the prevention of paratuberculosis (McAloon *et al.*, 2015).

Given the importance of colostrum bacterial levels, several researchers have attempted to provide benchmarking estimates of contamination levels at a national level. Previous thresholds for classification of bacterial contamination have been suggested at >100,000

colony-forming units (cfu) per ml and >10,000 cfu/ml for total bacterial counts (TBC) and coliform counts (CC) respectively (McGuirk and Collins, 2004). North American studies have reported 35.9% of samples exceeding TBC thresholds in Canada (Fecteau *et al.*, 2002), and 85% in the US (McGuirk and Collins, 2004), with later studies of 67 farms in 12 states finding 43% and 16.9% samples exceeding TBC and CC thresholds respectively (Morrill *et al.*, 2012). Only 18% of 255 samples from 44 Columbian herds were found to fail on TBC (Guzmán *et al.*, 2020). Of 268 NZ samples taken of pooled colostrum, 91.4% and 91.1% failed for TBC and CC respectively (Denholm *et al.*, 2017). Of 221 Australian colostrum samples, 41.6% and 27.6% of samples failed on TBC and CC respectively, with only 19.5% meeting both standards for immunoglobulin (>50g/L) and microbiological quality (Abuelo *et al.*, 2019), reinforcing previous Australian studies which found 42% and 6% of samples exceeded TBC and CC respectively, with only 23% meeting all standards for TBC, CC and immunoglobulin levels (Phipps *et al.*, 2016). Previous studies examining colostrum bacteriological levels in Irish dairy herds reported 56.5% of 214 samples exceeding TBC and 32.7% exceeding CC thresholds, with significant variation between farms (McAloon *et al.*, 2016), however, there have been no published studies on colostrum hygiene levels on GB dairy farms to date. In addition to a paucity of information around current GB colostrum bacterial levels, there is also a lack of knowledge around specific factors that may influence the bacterial contamination of colostrum (Phipps *et al.*, 2016).

The source of bacteria present in colostrum includes the mammary gland itself as well as contamination or proliferation during harvesting, storage or feeding (Fecteau *et al.*, 2002; McGuirk and Collins, 2004; Stewart *et al.*, 2005). Previous research has shown that colostrum contamination is generally extremely low when collected directly from the gland, with significant bacterial contamination occurring during the harvest process, suggesting steps to prevent colostrum contamination should focus largely on collection methods (Stewart *et al.*, 2005). Storage method also has an effect on bacterial levels, with colostrum stored at warmer conditions (22C) having >42 times more bacteria present and resulting in a serum IgG concentration almost twice as low compared with colostrum either pasteurized, untreated or stored at 4C for 2d (Cummins *et al.*, 2017), and bacterial levels have been shown to be significantly reduced when freeze-thawing colostrum (Issa Alrabadi, 2015). Irrespective of the source of colostrum contamination, it has been found that heat treatment is associated with reduced bacterial levels, improved health status and decreased mortality, even when receiving appropriate colostrum volume (Armengol and Fraile, 2016). This reinforces previous findings that calves fed heat-treated colostrum have significantly higher serum IgG concentrations, and a significantly lower risk of diarrhoea than those fed fresh colostrum (Godden *et al.*, 2012), suggesting colostrum pasteurisation may be an effective

method of reducing colostrum contamination levels. Whilst colostrum pasteurisation is likely to be effective at reducing colostrum bacterial levels, it is unknown how many GB farms currently pasteurise colostrum.

Whilst there are many farm level factors associated with colostrum contamination levels, it is likely that protocols aiming to prevent colostrum contamination are likely to vary between farms (McAloon *et al.*, 2016), and it is important that veterinary advisors are able to recommend interventions that are relevant to the majority of farms. The relative importance of management factors is essential for optimal decision making on-farm (Lima *et al.*, 2020). Bootstrapping allows for the estimation of robust coefficients (Breiman, 1996) and estimates of variable stability (Meinshausen and Bühlmann, 2010): an estimate of the reproducibility of a variable effect within a target population. The use of stability selection alongside regression techniques has been utilised in studies identifying a relatively small number of variables having a large and consistent effect on animal health outcomes (Lima *et al.*, 2020), and the identification of these variables can provide a succinct number of practical recommendations for veterinarians.

This study aims to provide a current benchmark of colostrum bacterial levels, investigate factors associated with increased levels of bacterial contamination of colostrum on GB dairy farms and provide practical recommendations for a small number of factors found to have the largest effect on colostrum hygiene on the largest number of farms.

4.2. Materials and methods

4.2.1. Farm selection

Farms recruited during research described in Chapter 3 were visited between 17th December 2018 – 14th February 2019 and provided with a colostrum collection kit and training on the collection and posting of colostrum samples. An additional 31 farms were enrolled by the same methodology (for inclusion in research described in Chapter 5) and were visited between 6th December 2019 – 29th January 2020 and were also provided with colostrum collection kits and training on collection protocols.

4.2.2. Colostrum collection protocol

Farmers were encouraged to take six colostrum samples from as close to the calf feeding point as possible, for example farms bottle-feeding colostrum to all calves should take samples from the bottle teat. Where colostrum feeding involved multiple sources, farmers were asked to collect samples from all sources, for example farms where calves would suckle colostrum from the dam in most cases and receive supplementary colostrum by

oesophageal tube feeding when necessary, should take some samples from the cows' teat, and some from the oesophageal tube. Farmers were asked to freeze samples as soon as possible after collection, with all six samples being collected within one month of each other. Sample pots contained glycerol as a cryopreservative. After collecting six samples, farmers were asked to place the samples in a pre-packaged insulated box containing ice packs provided to the farmer. These boxes were couriered to the laboratory (Quality Milk Management Services, Wells UK) for analysis.

4.2.3. Microbiological analysis

Samples were analysed using standard laboratory methods for milk (National Mastitis Council, 1999) as previously described (Bradley *et al.*, 2007). Ten microlitres of secretion was inoculated onto sheep blood agar and Edwards agar and 100µl was inoculated onto MacConkey agar. Total bacterial count and coliform counts were estimated after incubation for 72hr at 30C and 37C respectively.

4.2.4. Colostrum collection questionnaire

A submission form was submitted alongside colostrum samples including the cow id, date of sampling and sample collection location. Upon receiving the colostrum samples, farmers were contacted via telephone and asked about colostrum collection and equipment cleaning protocols used to collect the colostrum samples, such as the products used to clean equipment and whether hot water (defined as "scalding"; too hot to put their hand in) was used for cleaning. Where samples were taken directly from the cows' teat, the method used to clean collection buckets or feeding equipment was deemed irrelevant, and a "Not applicable" factor level was created. Similarly, where samples collected from the collection bucket but before using any feeding equipment, the methods used to clean feeding equipment was deemed irrelevant and a "Not applicable" factor level was created. The percentage of calves receiving manually fed colostrum feeds (feeds by bottle or tube as opposed to suckling from the dam) and the volume of first feeds was also recorded.

4.2.5. Descriptive analysis

All data analysis was conducted in R (R Core Team, 2020). The percentage of colostrum samples failing in terms of total bacteria count (TBC) and coliform count (CC) was calculated for each farm, with a "failure" being when >100,000 for TBC and >10,000 for CC as previously suggested by McGuirk and Collins (2004).

4.2.6. Statistical analysis

Both TBC and coliform counts were natural log transformed after the addition of 1 to all counts. Samples with missing data were removed from the dataset. Continuous variables were scaled (divided by one standard deviation) and centred prior to modelling using the *preProcess* function within the *caret* package (Kuhn, 2018). Categorical variables were releveled to include as few relevant categories as possible.

For model building, a bootstrap sample was taken from the dataset (sampling with replacement to create a sample of the same size as the original dataset). A mixed model was created from the bootstrapped data sample using the *lmer* function from the *lmerTest* package (Kuznetsova, Brockhoff and Christensen, 2017) with TBC or CC as model outcomes in respective models. Farm was included as a random effect, and all other colostrum management variables were included as fixed effects as shown in Table 4-1. The following model equation was used for the mixed model

$$Y_{ij} = \mu + \beta_1 X_{1ij} + \beta_2 X_{2ij} \ldots + U_j + \epsilon,$$

where Y_{ij} is the log TBC or CC of the *i*th sample on the *j*th farm. X_{1ij} represents covariates at the sample farm level, with corresponding coefficients represented by β_1, and X_{2ij} representing covariates at the sample farm level, with corresponding coefficients represented by β_2. μ represents the intercept, β represents explanatory variables, U_j as the farm specific random effect for the *j*th farm, and ϵ as the random error. The assumed distributions of U and ϵ are normal, with mean zero and variance $\theta\, U$ and $\theta\, \epsilon$ respectively.

An automated backwards stepwise selection process based on Akaike information criterion was used using the step function from the *lmerTest* package (Kuznetsova, Brockhoff and Christensen, 2017) to create a final mixed effects model for a given bootstrap sample, and variables from the final model were recorded alongside coefficient values. This process was then repeated 1,000 times, recording the presence of variables and their corresponding effect size in each iteration. Residuals were checked to ensure near normal distribution after building an automated backwards stepwise mixed effects model on the full dataset (i.e., without bootstrap sampling).

Variable stability was calculated as the percentage of bootstrap models in which a given variable was selected. Mean coefficient values and 95% bootstrap confidence intervals (BCI) were calculated from coefficient values across all bootstrap samples in which a variable was selected. An estimate of significance as a "bootstrap p-value" was calculated as one minus the proportion of coefficient estimates on the majority side of zero (proportion below zero if the mean coefficient was above zero, and proportion above zero if mean coefficient was

above zero). Variables with a bootstrap stability >10% and a bootstrap p-value <0.025 were deemed to be both relatively stable and have reasonable effect size.

Variable	Levels
Sample collection point	Cows teat, Colostrum collection bucket, Feeding teat, Oesophageal tube
Number of days between calving pen clean out	Numeric
Pre-milking teat disinfection used	No, Yes
Teat dry wiped prior to colostrum collection	No, Yes
Milking system	Parlour, Robot, Not applicable
Frequency of colostrum collection equipment cleaning	Each use, Less than each use, Not applicable
Method of colostrum collection equipment cleaning	Water, Hypochlorite, Parlour wash, Peracetic acid, Soap, Not applicable
Hot water used to clean collection equipment	No, Yes, Not applicable
Frequency of colostrum feeding equipment cleaning	Each calf, Less than each calf, Not applicable
Method of colostrum feeding equipment cleaning	Water, Hypochlorite, Parlour wash, Peracetic acid, Soap, Not applicable,
Hot water used to clean feeding equipment	No, Yes, Not applicable
Colostrum frozen prior to sample collection	No, Yes, Not applicable
Colostrum pasteuriser used	No, Yes, Not applicable

Table 4-1: Colostrum management variables and factor levels available as fixed effects for model building.

4.3. Results

A total of 356 samples were returned from 59 farms. Fifteen samples from six farms were either missing or damaged in transit and were removed from the dataset. Thirteen samples from 3 farms were removed due to missing or incomplete data on sample collection. Thirty-two farms failed to return any samples, with 19 farms failing to return samples from the first round of data collection, and 13 farms failing to return samples during the second round of data collection. The final dataset consisted of 328 samples from 56 farms. One hundred and fifty-one samples were collected from feeding equipment, with 80 (53.0%) being collected through a feeding teat and 71 (47.0%) being collected through an oesophageal feeding tube. One hundred and nineteen samples were collected from a collection bucket and 58 samples were collected directly from the cow's teat. Pre-milking teat disinfection was used prior to colostrum collection for 271 (82.6%) samples compared with 57 (17.4%) samples where no pre-milking disinfection was used. Dry wiping of teats prior to colostrum collection was

conducted for 270 (82.3%) samples compared with 58 (17.7%) with no dry wiping of teats. The frequency of the cleaning out of calving pens was between 3.5d and 90d, with a mean and median of 27.9d and 28d respectively. Farmers reported manually feeding colostrum (by bottle or tube as opposed to suckling from the dam) to between 0% and 100% of calves, with a mean and median of 79.2% and 100% respectively. Colostrum volume fed by farmers at first feed ranged from 2 to 5L at first feed, with a mean and median of 3.1L and 3.0L respectively.

Of the 270 samples collected using milking equipment (i.e., excluding the 58 samples collected directly from the teat), 220 (81.5%) were collected through a milking parlour, and 50 (18.5%) through a robotic milking unit. Only 69 samples (25.6%) were submitted from farms where collection equipment (i.e., collection bucket) was cleaned after each use, with 201 (74.4%) being collected from farms where collection equipment was cleaned less frequently than after each use. Seventy-nine samples (29.2%) were collected from farms using water to clean collection equipment, 94 (34.8%) using parlour washings, 54 (20.0%) from farms using hypochlorite, 31 (11.5%) using peracetic acid, and 12 (4.4%) using soap. One hundred and forty-five samples (53.7%) were collected from farms using hot water to clean collection equipment, compared with 125 (46.3%) from farms that did not use hot water. Farms that used a colostrum pasteuriser accounted for 11 samples (4.1%), compared with 259 samples (95.9%) where a pasteuriser was not used. Colostrum was frozen prior to sample collection for 14 samples (5.2%) compared with 256 samples (94.8%) which were collected without prior freezing.

Of the 151 samples collected directly from feeding equipment (i.e., excluding the 58 samples collected directly from the teat and the 119 samples collected from the collection bucket) 102 samples (67.5%) were collected from farms where feeding equipment was cleaned every time it was used, and 49 samples (32.4%) when feeding equipment was cleaned less than each use. Thirty-four samples (22.5%) were collected from farms using water alone to clean feeding equipment, 24 (15.9%) using parlour washings, 45 (29.8%) from farms using hypochlorite, 24 (15.9%) using peracetic acid, and 24 (15.9%) from farms using soap. One hundred and two samples (67.5%) were from farms that used hot water to clean feeding equipment, compared with 49 (32.4%) from farms that did not use hot water.

Mean TBC and Coliform counts were 326,931 cfu/ml and 13,034 cfu/ml with median values of 14,800 cfu/ml and 1 cfu/ml respectively. Ninety-seven (29.6%) samples had TBC results above threshold (>100,000 cfu/ml) and 25 samples (7.6%) had coliform counts above threshold (>10,000 cfu/ml).

Mean and median TBC were lower when samples were collected directly from the cow's teat, at 32,079 and 535 respectively, with only 6.9% of samples being above threshold, compared with collection from a collection bucket (mean 327,879, median 44,000, 35.3% above threshold) or feeding equipment (mean 439,438, median 18,100, 33.8% above threshold). Coliform counts were also lower when samples were collected from the cow's teat (mean 21, median 0, 0.0% above threshold) compared with samples taken from the collection bucket (mean 13,294, median 2, 7.6% above threshold) or feeding equipment (mean 17,859, median 1, 10.6% above threshold). A higher number of samples collected directly from the cow's teat had zero coliforms present, with 43 samples (74.1%) having zero coliforms when collected directly from the cow's teat compared with 48 samples (40.3%) taken from the collection bucket and 62 samples (41.0%) when collected from feeding equipment. In contrast, only one sample collected directly from a cows' teat, and zero samples collected from collection or feeding equipment had a zero TBC. A lower proportion of samples collected from cows' teats (6.9%) were above threshold for either TBC or CC than those collected from collection buckets (37.0%) or feeding equipment (34.4%).

Thirteen variables were available for predicting both colostrum TBC and CC. Variables with a stability >10% and a bootstrap p-value <0.025 were included in the final models resulting in 8 and 7 variables being selected for TBC and CC respectively.

4.3.1. Total bacterial counts

The use of a milking machine was associated with an increase in TBC compared with those collected directly from the cows' teat with a stability of 92.5% being associated with a 2.06 log cfu/ml (95% BCI: 0.35 - 3.71) increase when collected through a parlour, and a 3.38 log cfu/ml (95% BCI: 1.29 - 5.80) increase when collected through a robot. Sample collection point was also associated with increased TBC, with a stability of 87.3%, being associated with a 2.36 log cfu/ml (95% BCI: 0.77 - 5.45) increase when collected from feeding equipment compared with samples collected directly from cows' teats.

The use of hot water to clean feeding equipment was associated with reduced TBC with a stability of 85.9%, being associated with a -2.54 log cfu/ml (95% BCI: -3.76 - -1.74) reduction when hot water was used. The method of cleaning colostrum collection buckets was associated with TBC with a stability of 29.1%. Compared with water, cleaning colostrum collection buckets with peracetic acid was associated with a -2.04 log cfu/ml (95% BCI: -3.49 - -0.56) reduction in TBC, and a tendency towards reduced TBC was identified when cleaning with hypochlorite (-1.60 log cfu/ml, 95% BCI: -3.01 - 0.27) and soap (-1.14 log cfu/ml, 95% BCI: -3.01 - 0.27). No difference was detected when cleaning

with parlour wash (0.47 log/cfu/ml, 95% BCI: -0.76 - 1.89) compared with water. The frequency of colostrum collection equipment cleaning was associated with TBC with a stability of 22.1%, and a 1.75 log cfu/ml (95% BCI: 1.30 - 2.49) increase when collection equipment was cleaned less than every time it was used. The wiping of teats prior to colostrum collection was associated with a reduction in TBC with a stability of 23.3% and a -1.97 log cfu/ml (95% BCI: -2.85 - -1.45) reduction in TBC. The use of a colostrum pasteuriser was associated with TBC with a stability of 10.9% and a -3.79 log cfu/ml (95% BCI: -5.87 - -2.93) reduction in TBC.

Variables with >10% stability and <0.025 bootstrap p-value are depicted in Figure 4-1, with stability estimates of variables being presented in Figure 4-2. Coefficients, 95% BCI and stability estimates for all model variables are presented in Table 4-2.

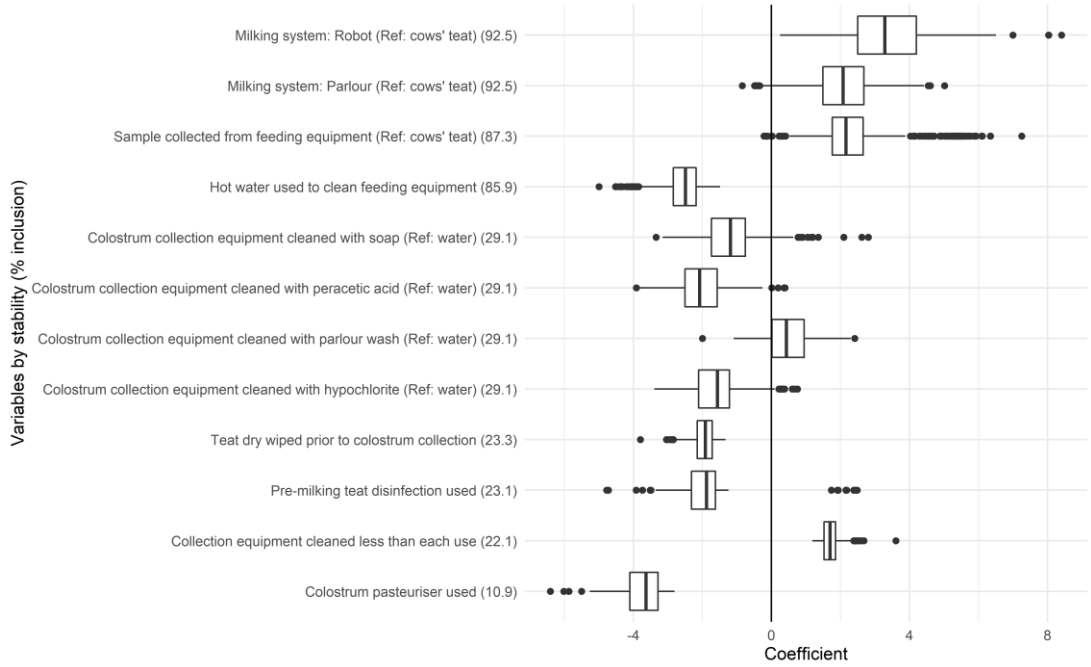


Figure 4-1: Coefficient distributions and variable stability for variables selected in at least 10% of models across 1,000 bootstrapped samples. Coefficient estimates represent the change in total bacterial count (log cfu/ml).

Variable	N	Stability (%)	Mean coefficient	95% Bootstrap confidence interval	Bootstrap P-value
Milking system: Parlour (Ref: cows' teat)	220	92.5	2.06	(0.35 - 3.71)	0.01
Milking system: Robot (Ref: cows' teat)	50	92.5	3.38	(1.29 - 5.80)	<0.01
Sample collected from feeding equipment (Ref: cows' teat, n=58)	151	87.3	2.36	(0.77 - 5.45)	0.01
Hot water used to clean feeding equipment	102	85.9	-2.54	(-3.76 - -1.74)	<0.01
Colostrum collection equipment cleaned with hypochlorite (Ref: water)	54	29.1	-1.60	(-3.01 - 0.27)	0.04
Colostrum collection equipment cleaned with parlour wash (Ref: water)	94	29.1	0.47	(-0.76 - 1.89)	0.24
Colostrum collection equipment cleaned with peracetic acid (Ref: water)	31	29.1	-2.04	(-3.49 - -0.56)	0.01
Colostrum collection equipment cleaned with soap (Ref: water)	12	29.1	-1.14	(-2.55 - 1.13)	0.10
Teat dry wiped prior to colostrum collection	270	23.3	-1.97	(-2.85 - -1.45)	<0.01
Pre-milking teat disinfection used	271	23.1	-1.85	(-3.39 - 2.23)	0.05
Collection equipment cleaned less than each use	201	22.1	1.75	(1.30 - 2.49)	<0.01
Colostrum pasteuriser used	11	10.9	-3.79	(-5.87 - -2.93)	<0.01
Feeding equipment cleaned less than each calf	49	9.7	-2.13	(-2.98 - -1.63)	<0.01
Hot water used to clean collection equipment	145	9.1	-1.60	(-2.17 - -1.16)	<0.01
Sample collected from collection equipment (Ref: cows' teat)	119	6.4	3.51	(2.36 - 4.38)	<0.01
Colostrum feeding equipment cleaned with hypochlorite (Ref: water)	45	6.3	3.23	(0.25 - 5.29)	0.03
Colostrum feeding equipment cleaned with parlour wash (Ref: water)	24	6.3	2.66	(0.96 - 4.47)	<0.01
Colostrum feeding equipment cleaned with peracetic acid (Ref: water)	24	6.3	3.84	(1.34 - 5.75)	0.02
Colostrum feeding equipment cleaned with soap (Ref: water)	24	6.3	2.29	(0.09 - 3.83)	0.03
Number of days between calving pen clean out	328	3.3	0.65	(0.54 - 0.84)	<0.01
Colostrum frozen prior to sample collection	14	2.3	0.75	(-2.53 - 3.34)	0.35
Colostrum collection equipment cleaning: Not applicable	58	1.1	-4.03	(-4.64 - -3.48)	<0.01

Table 4-2: Stability, mean coefficient (log cfu/ml), 95% bootstrap confidence intervals and bootstrap p-value for all variables associated with total bacterial count. N represents the number of samples where variable was "positive".

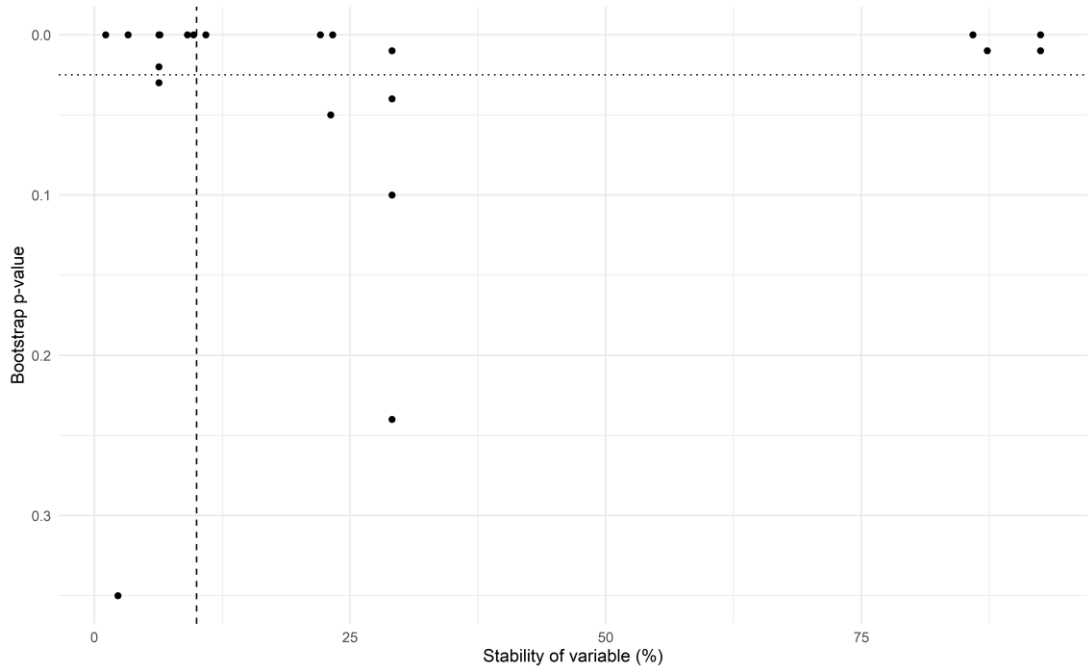


Figure 4-2: Bootstrap p-value by stability of variables for total bacterial counts. Variables were selected for final model were above 10% stability (dashed line) with a bootstrap p-value of <0.025 (dotted line).

4.3.2. Coliform counts

The use of hot water to clean both feeding equipment and collection equipment was associated with CC, with stabilities of 51.9% and 32% respectively. The use of hot water to clean feeding equipment and collection equipment was associated with a -2.72 log cfu/ml (95% BCI: -4.01 - -1.82) and -1.72 log cfu/ml (95% BCI: -2.35 - -1.26) reduction in CC respectively. The sample collection point was associated with CC with stabilities of 45.6% and 45% when samples were collected from feeding equipment or collection equipment compared with directly from the cows' teat. Collection of samples from feeding equipment was associated with a 3.40 log cfu/ml increase in CC (95% BCI: 1.26 - 5.59), and a tendency for increased CC was identified when samples were collected from collection equipment (1.49 log cfu/ml, 95% BCI: -0.28 - 3.03) compared with samples collected directly from the cows' teat.

The method of colostrum collection equipment cleaning was associated with CC with a stability of 19.9%. Compared with using water, the use of peracetic acid was associated with a -1.66 log cfu/ml (95% BCI: -2.73 - -0.54) reduction in CC and the use of parlour wash was associated with a 1.28 log cfu/ml (95% BCI: 0.05 - 2.46) increase in CC. Hypochlorite tended to decrease CC (-0.64 log cfu/ml, 95% BCI: -2.29 - 0.75) and no difference was found when soap was used (0.03 log cfu/ml, 95% BCI: -2.41 - 3.06). The cleaning of

colostrum collection equipment less frequently than every use was associated with a 1.68 log cfu/ml (95% BCI: 1.19 - 2.18, stability 17.9%) increase in CC, and the wiping of teats prior to colostrum collection was associated with a -2.33 log cfu/ml (95% BCI: (-3.46 - -1.53, stability 11.4%) reduction in CC.

Variables with >10% stability and <0.025 bootstrap p-value are depicted in Figure 4-3, with stability estimates of variables being presented in Figure 4-4. Coefficients, 95% BCI and stability estimates for all model variables are presented in Table 4-3.

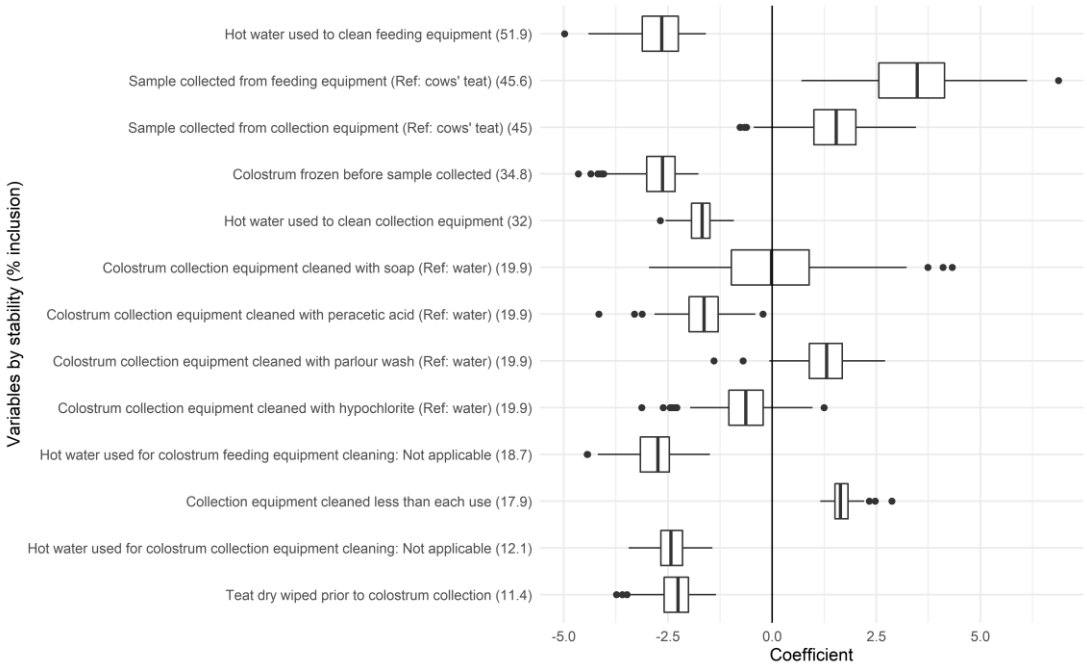


Figure 4-3: Coefficient distributions and variable stability for variables selected in at least 10% of models across 1,000 bootstrapped samples. Coefficient estimates represent the change in coliform count (log cfu/ml).

Variable	N	Stability	Mean coefficient	95% Bootstrap confidence interval	Bootstrap P-value
Hot water used to clean feeding equipment	102	51.9	-2.72	(-4.01 - -1.82)	<0.01
Sample collected from feeding equipment (Ref: cows' teat)	151	45.6	3.40	(1.26 - 5.59)	<0.01
Sample collected from collection equipment (Ref: cows' teat)	119	45	1.49	(-0.28 - 3.03)	0.05
Colostrum frozen prior to sample collection	14	34.8	-2.70	(-3.93 - -1.92)	<0.01
Hot water used to clean collection equipment	145	32	-1.72	(-2.35 - -1.26)	<0.01
Colostrum collection equipment cleaned with hypochlorite (Ref: water)	54	19.9	-0.64	(-2.29 - 0.75)	0.15
Colostrum collection equipment cleaned with parlour wash (Ref: water)	94	19.9	1.28	(0.05 - 2.46)	0.02
Colostrum collection equipment cleaned with peracetic acid (Ref: water)	31	19.9	-1.66	(-2.73 - -0.54)	<0.01
Colostrum collection equipment cleaned with soap (Ref: water)	12	19.9	0.03	(-2.41 - 3.06)	0.52
Hot water used for colostrum feeding equipment cleaning: Not applicable	177	18.7	-2.82	(-4.15 - -2.00)	<0.01
Collection equipment cleaned less than each use	201	17.9	1.68	(1.19 - 2.18)	<0.01
Hot water used for colostrum collection equipment cleaning: Not applicable	58	12.1	-2.40	(-3.32 - -1.64)	<0.01
Teat dry wiped prior to colostrum collection	270	11.4	-2.33	(-3.46 - -1.53)	<0.01
Colostrum frozen prior to sample collection: Not applicable	58	9.6	-0.63	(-1.90 - 0.13)	0.10
Pre-milking teat disinfection used	271	8	1.99	(1.46 - 2.70)	<0.01
Colostrum collection equipment cleaning: Not applicable	58	7.8	-1.89	(-2.82 - -0.84)	<0.01
Colostrum pasteuriser used	11	7	-3.89	(-5.36 - -3.13)	<0.01
Collection equipment cleaned less than each calf	201	4.7	-2.13	(-2.57 - -1.73)	<0.01
Colostrum feeding equipment cleaned with hypochlorite (Ref: water)	45	2.1	-0.78	(-4.47 - 1.99)	0.33
Colostrum feeding equipment cleaned with parlour wash (Ref: water)	24	2.1	-0.17	(-3.96 - 3.49)	0.43
Colostrum feeding equipment cleaned with peracetic acid (Ref: water)	24	2.1	-0.76	(-3.85 - 0.57)	0.24
Colostrum feeding equipment cleaned with soap (Ref: water)	24	2.1	3.13	(-2.72 - 5.37)	0.10
Milking system: Parlour (Ref: cows' teat)	220	1.4	1.75	(0.57 - 3.23)	<0.01
Milking system: Robot (Ref: cows' teat)	50	1.4	2.18	(0.40 - 3.97)	<0.01
Number of days between calving pen clean out	328	0.4	0.68	(0.65 - 0.74)	<0.01

Table 4-3: Stability, mean coefficient (log cfu/ml), 95% bootstrap confidence intervals and bootstrap p-value for all variables associated with coliform count. N represents the number of samples where variable was "positive".

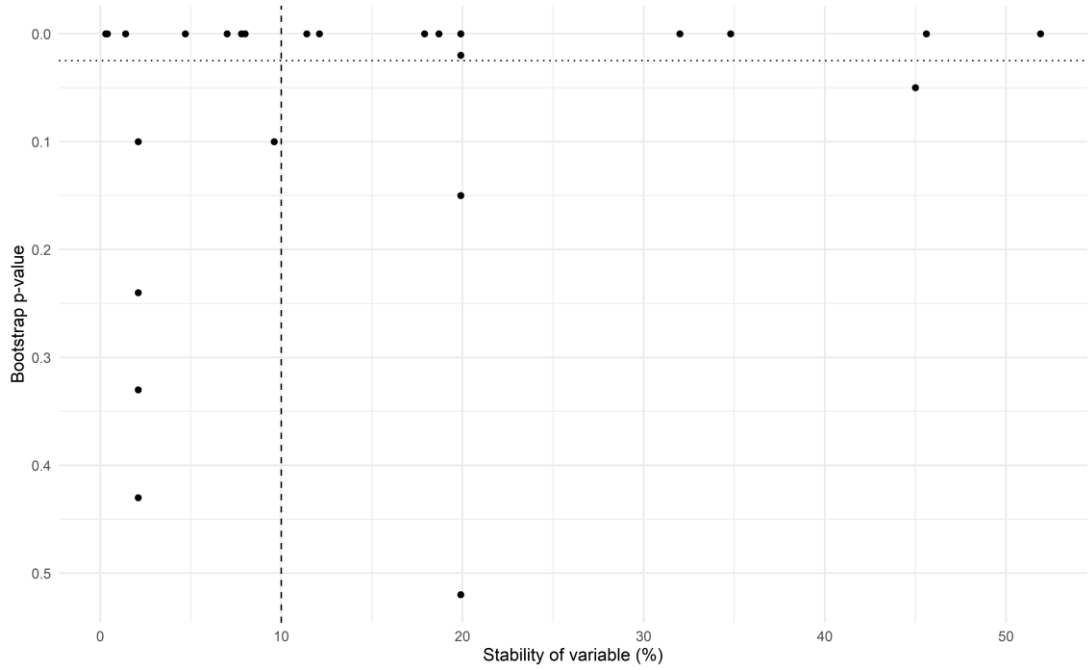


Figure 4-4: Bootstrap p-value by stability of variables for coliform counts. Variables were selected for final model were above 10% stability (dashed line) with a bootstrap p-value of <0.025 (dotted line).

As the interpretation of natural log coefficients are not necessarily intuitive, a conversion to log10 (by dividing by 2.303) and subsequently the percent reduction in TBC to be expected when implementing the most stable and significant variables identified is shown in Table 4-4.

Variable	Natural log coefficient	Log10 coefficient conversion	Percent reduction in TBC (%)
Feeding equipment cleaned after each calf	-1.75	-0.76	83
Colostrum collection equipment cleaned with hypochlorite or peracetic acid (Ref: Water)	-2	-0.87	86
Hot water used to clean feeding equipment	-2.54	-1.10	92
Premilking teat disinfection used	-1.85	-0.80	84
Pasteuriser used	-3.79	-1.65	98

Table 4-4: Coefficient conversion and percent reduction in total bacterial count (TBC) for factors most likely to have both a large effect size on the majority of farms.

4.4. Discussion

This is the first study to report TBC and CC in colostrum samples from GB dairy farms and provides and initial benchmark of colostrum hygiene. Samples collected directly from cows' teats had relatively low levels of bacterial contamination with only 6.9% of samples being above threshold for either TBC or CC, compared with 37.0% and 34.4% of samples collected from collection and feeding equipment, respectively. This suggests that bacterial contamination is not likely to originate from the cow, and rather from the milking machine, collection buckets and feeding equipment as has been previously suggested (Stewart *et al.*, 2005).

Several variables were identified as having both relatively high stability and having a relatively large effect size in reducing TBC and/or CC in colostrum. The use of a milking machine to harvest colostrum was associated with increased bacterial counts, and samples from both collection and feeding equipment were associated with higher bacterial counts compared with those collected directly from the cows' teat. This large and consistent effect size for both TBC and CC suggests interventions should be targeted primarily at equipment hygiene protocols. The use of hot water was found to have a relatively large effect on bacterial counts, with samples collected from both collection equipment and feeding equipment cleaned with hot water being associated with significantly lower bacterial counts than those where cold water was used. In addition to a large effect size, these were relatively stable variables, suggesting that interventions focused on these variables would have a substantial effect on a large number of farms. As hot water was often not used to clean equipment for collection (46.3% of samples) or feeding (32.4% of samples) equipment, this represents an easy intervention for veterinarians to target on a large number of farms that could have a substantial and immediate impact on colostrum hygiene for GB dairy farms.

Disinfection of collection equipment with either hypochlorite or peracetic acid was found to have a relatively large effect size and high stability for reducing both TBC and CC. Only 31.5% of samples were collected from farms using hypochlorite of peracetic acid to clean colostrum collection equipment, with the remaining farms predominantly using either parlour washings or water. Given the large number of farms following ineffective disinfection protocols this again represents a relatively straightforward intervention to target on the majority of farms. The use of a colostrum pasteuriser was consistently associated with reduced TBC and CC, with a large effect size relative to other variables. The stability however was relatively low, at 10.9% and 7.0% for TBC and CC respectively, suggesting that whilst colostrum pasteurization is likely to have a large effect size in reducing TBC and CC in a small number of cases, it does not seem to have a significant effect in many of the

bootstrap samples taken from the original dataset. This is likely due to the infrequent use of pasteurisation equipment in this sample, with only 4.1% of samples collected from farms using a pasteuriser and therefore many bootstrap samples will not contain any samples collected after pasteurisation. Whilst colostrum pasteurisation appears to have a relatively large effect size in reducing TBC and CC, the relative scarcity of colostrum pasteurisation and the requirement for initial financial investment may make this a more challenging intervention for veterinarians to implement on a large number of farms.

Based on the results from this trial, practical recommendations for veterinary intervention should focus on the effective cleaning of colostrum collection and feeding equipment after every use with hot water as opposed to cold water, and hypochlorite or peracetic acid as opposed to water or parlour wash. Cows' teats should be prepared with a pre-milking teat disinfectant and wiped with a clean, dry paper towel prior to colostrum collection, and colostrum should be pasteurised where possible. Only 23 samples in the current dataset were collected from farms following the optimal collection practices identified in this study (cleaning both collection and feeding equipment each time they were used with hot hypochlorite or peracetic acid and using a pre-milking teat disinfection and dry wipe prior to collection, but excluding pasteurisation). By following these simple recommendations, it is likely that significant reductions in both TBC and CC will be achieved, although these results should be validated in a randomised controlled trial in future research.

Pasteurising colostrum is likely to be effective at reducing TBC and CC as described previously (Godden *et al.*, 2012; Gelsinger, Jones and Heinrichs, 2015), however, given the relatively high financial investment required for pasteurisation equipment, other interventions might be considered first where economic constraints apply. Whilst factors such as colostrum pasteurisation might have a large impact on colostrum hygiene (relatively large effect size), this might only be applicable to a small number of farms (low stability). Similarly, some factors are applicable to a large number of farms (high stability) however only have a small impact on colostrum hygiene (relatively small effect size). The recommendations from this trial are not intended to provide an exhaustive list of all factors affecting colostrum hygiene, rather identify a small number of practically implementable interventions that had the largest effect size on the majority of farms. Whilst there are several key variables identified in this research, it is likely that there are other variables that also impact colostrum bacterial levels to some degree.

Whilst TBC and CC are highly correlated, it has been suggested that CC might be a better predictor of disease. Whilst a threshold of CC <10,000 cfu/ml is a reasonable threshold to aim for, the negative linear relationship between CC and serum IgG suggests there is no

optimal threshold, and it may be better to aim for as low as possible (Godden *et al.*, 2012). A CC target of as low as possible would be supported by the current trial, with the 41% of samples from feeding equipment having zero CC suggesting that 0 cfu/ml is an achievable target for coliforms in colostrum from GB dairy producers.

Whilst all milking machine use was associated with higher levels of bacterial contamination, robot milking machines were associated with a particularly high level of contamination. This may be due to default settings for the collection of colostrum by the robot rather than any inherent issue with robot systems themselves. Although the default setting for robots participating in this trial for routine milk collection was generally to perform a full machine wash and perform pre-milking teat cleaning prior to milking, these were often not performed prior to the collection of first colostrum. Veterinarians on robotically milked dairy farms should investigate default colostrum collection settings and ensure that settings are configured for optimal colostrum hygiene.

Farmer collection of colostrum samples represents a potential limitation of this study, as there is likely to be a degree of inconsistency in sampling technique. It is likely, however, that calves being fed colostrum experience a similar degree of inconsistency in hygiene practices, and therefore the results from this trial are likely to be relatively indicative of bacterial levels experienced by calves on GB dairies. Variability in sample collection technique between farmers is likely to be random and is unlikely to introduce bias into models. It is possible that by being enrolled in a trial, an element of bias may have been introduced with farmers being keen to process colostrum in a relatively hygienic manner. The simple act of benchmarking has been reported to decrease levels of FPT from 21% to 11% after a benchmark report and change in management (Atkinson, von Keyserlingk and Weary, 2017), although every effort was made to encourage farmers to collect samples as normal. This bias was limited by the design of the study to some degree, as farmers were only asked about collection protocols used for the samples after the samples were received. As enrolment in the trial was voluntary it is possible that farmers on this trial represent a more progressive population, and therefore estimates of bacterial contamination levels are likely to be conservative. Concurrent research was also being undertaken on the study farms (as described in Chapter 5) which may have introduced a level of bias, particularly an intervention trial aimed at increasing growth rates in preweaned calves. One component of this was encouraging farmers to use hypochlorite or peracetic acid when collecting colostrum, however, on further analysis, only 28 samples (8.5%) were from intervention farms where farmers were now using a recommended cleaning product where they were not previously, compared with 15 samples (4.6%) from control farms. Differences between control and intervention groups were not significant after performing a chi-squared test

(p=0.16), and the authors feel that whilst this may have had a small effect on the numbers of farms using hypochlorite or peracetic acid overall, this is unlikely to have a significant effect on bacterial estimations and have no effect on model performance or recommendations from this trial.

Stability of variables in predicting coliform counts were far lower than TBC. Log transforming total bacterial counts resulted in a gaussian distribution for TBC. Due to a large number of zero counts, however, CC did not fit a gaussian distribution after log transformation. The distribution of residuals for CC models were carefully checked however and were deemed to provide satisfactory evidence of model fit. Furthermore, any prediction errors at extreme values are likely to be ameliorated by the bootstrapping process. The use of regularised regression models was also considered due to their effective performance in robust variable selection (Zou and Hastie, 2005), however, due to the presence of multiple samples from each farm and relatively few explanatory variables, mixed-effects models were better suited to the dataset. Whilst the recommendations from this research are likely to be applicable to dairy farms in GB, caution should be taken when extrapolating the results to dairy farms in other countries.

4.5. Conclusions

Colostrum sampled from collection or feeding equipment had higher levels of TBC and CC than those taken directly from cows' teats suggesting microbiological contamination is likely to occur from improperly cleaned equipment rather than the cow. Whilst extremely low bacterial counts were achievable, this study indicates over one third of samples collected from either collection buckets or feeding equipment were over conventional thresholds for either TBC or CC, and would, therefore, represent a significant risk for both the ingestion of pathogens and the failure of passive transfer of immunity on GB dairy farms.

Routine testing of colostrum bacteriology is relatively cheap and straightforward and is likely to be currently underutilised in the UK. Veterinarians should consider routine colostrum hygiene testing as part of a preventative calf health approach, and this trial has identified a small number of variables that are likely to have a substantial impact on colostrum hygiene for a large proportion of farms. Key recommendations based on this research to reduce bacterial levels in colostrum suggest protocols should include the cleaning of colostrum collection and feeding equipment after every use with hot water as opposed to cold water, and hypochlorite or peracetic acid as opposed to water or parlour wash. Cows' teats should be prepared with a pre-milking teat disinfectant and wiped with a clean, dry paper towel prior to colostrum collection, and colostrum should be pasteurised where possible.

To make the findings of this research accessible to farmers and practitioners, a “Colostrum bacteriology” tool has been added to the Nottingham University Herd Health Toolkit, where users can enter their current colostrum cleaning protocols and receive specific advice aiming to reduce bacterial levels within colostrum.

Chapter 5 Improving growth rates in preweaned calves on dairy farms, a randomised controlled trial

This chapter describes a randomised controlled trial aiming to test the impact of implementing specific management factors identified in previous chapters, through the implementation of a calf health plan compared with a negative control. A close derivative of this chapter has been submitted for publication in the Journal of Dairy Science.

5.1. Introduction

The health and performance of dairy and beef cross calves reared on dairy farms is important (Windeyer et al., 2014), both in terms of welfare (Renaud et al., 2018) and economics (Boulton et al., 2017; Mõtus et al., 2017). Recent research in the UK found that during the preweaning period, 48% of UK calves experienced diarrhoea and 46% experienced respiratory disease (Johnson et al., 2017). Significant variations in average daily gain (ADG) have also been reported in calves from UK dairy herds (Bazeley et al., 2016). Dairy farmers often rank youngstock health as a lower priority than the health of adult cows (Atkinson, 2015), and whilst the economic importance of female dairy replacement animals is well known (Brickell et al., 2009; Hultgren and Svensson, 2009; Boulton et al., 2017), previous research has suggested that male dairy calves may receive a lower standard of care than their female counterparts due to their relatively low economic value (Renaud et al., 2017). A major priority for dairy farms in Great Britain (GB), is to improve the growth and survival rate of youngstock and further increase the proportion of dairy bull calves entering the beef supply chain (CHAWG, 2017).

Previous studies have identified key farm management factors associated with calf morbidity including colostrum management practices, housing hygiene and level of veterinary involvement (Windeyer et al., 2014; Johnson et al., 2017; Renaud et al., 2018) as discussed in chapter 3, and several studies have estimated the effect of specific environmental conditions on mortality rates (Pannwitz, 2015; Santman-Berends *et al.*, 2021), and reinforced by research discussed in Chapter 2. Identifying key environmental and management factors associated with calf morbidity, mortality and productivity is essential for veterinarians and farmers to implement effective management changes. The research described in Chapter 3 resulted in the identification of several key environmental and management factors associated with increased ADG on British dairy farms, specifically environmental temperatures, housing hygiene and milk/colostrum feeding practices. Management factors identified in previous studies to improve calf health and productivity outcomes have not been tested in a randomised controlled trial on dairy farms; this is

essential to identify causal relationships and estimate the true effects of an intervention. It was hypothesized that a herd health plan involving these factors might be effective in improving calf health and production outcomes.

The objective of this study was to determine the effect of implementing an evidence-based calf health plan on both productivity and health outcomes for calves reared on dairy farms in GB.

5.2. Materials and methods

Sixty dairy farms in GB were recruited from a national supermarket supply group. Thirty-one of those farms were re-enrolled having previously been enrolled as described in Chapter 3. To recruit the remaining 29 farms that were required, 120 dairy farms were selected at random from the same national supermarket supply group. An informal letter was sent to the 114 farms with valid contact details and followed up with a telephone call through the farms in random order until the remaining 29 farms were recruited (92 farms contacted), resulting in a total of 60 farms recruited.

Farms were randomly allocated to one of two groups, either intervention (INT) or control (CON). Randomisation was performed using the *sample* function in R (R Core Team, 2020) after grouping farms by geographical region (North (n = 7), South (n = 25) or Midlands (n = 31)) and recruitment group (farms re-enrolled from the previous trial and newly recruited farms), where INT farms would receive a calf health plan, and CON farms would receive the health plan after 6-months. Blinding of the farmer and veterinarian providing the plan was not possible.

All farms were visited between 4th December 2019 and 29th January 2020 to conduct a calf management questionnaire and provide data collection training. During the initial visit, INT farms were provided with a calf health plan based on their responses to the management questionnaire, whereas CON farms received no calf health advice. Questionnaire responses were recorded separately for both male or beef calves (MB) and dairy heifer calves (DH) for all 20 questions.

The calf health plan provided to INT farms was created using data from research described in Chapter 3, where key housing and management variables were identified as having the potential for major improvements on ADG, morbidity or mortality on the majority of farms. The final plan consisted of 20 possible interventions deemed to have the greatest potential to improve ADG, morbidity and mortality rates. To determine which of the 20 interventions were appropriate for a given farm, the calf health plan was implemented using an online tool through the *shiny* platform (Chang et al., 2021). The tool allowed the researcher and farmer

to enter farm-specific management and housing information and be provided with a "traffic light" coloured representation of current management practices as well as an automated written report, providing bespoke information and suggestions based on the data provided. An automated "Agreed recommendations" sheet was provided, with the top 10 interventions recommended by the health plan software. A full list of the possible interventions is provided in Figure 5-1, and all questions and interventions are available through the herd health toolkit (www.nottingham.ac.uk/herdhealthtoolkit, under "Calf health plan", and in A*ppendix ii*). Farmers were encouraged to select and tick as many recommendations as they thought practical. Farmers were contacted at least monthly via phone call or SMS message throughout the trial period to encourage compliance with the implementation of the agreed interventions. An outline of the questionnaire themes is provided below.

Calving pen

This section included the proportion of cows calving in a dedicated calving pen and the frequency of calving pen clean out.

Colostrum

This section included questions relating to colostrum provision, including the length of time a calf might go without colostrum feeding, and how many litres would the calf receive in the first 6 hours of life. This section also contained questions on the method and frequency of colostrum equipment cleaning.

Feeding

This area covered what the calves are fed (milk replacer or whole milk), the volume and frequency of feed.

Housing

This section included the calf building design, including how much of it was covered by roof/sides, the frequency of clean out, and both the frequency of application and nesting potential of bedding. This set of questions also covered whether any calf shelters or heat sources were present within the shed, whether animals shared airspace with any weaned animals, and the age of calf at disbudding.

All farms received training in the use of the weigh tapes provided for the trial (MSD, Milton Keynes, UK) including a demonstration of their use during the farm visit and were instructed to record weights at birth and weaning for all calves born during the study period. To standardise the diagnosis of diarrhoea and pneumonia, farmers received training in the use of a version of the Wisconsin calf health scoring system (McGuirk, 2008) that had previously

been adapted for the UK (Johnson et al., 2017). Farmers were asked to record the date of the first case of diarrhoea or pneumonia and the date of any deaths that occurred during the trial period so that an incidence risk might be calculated (CDC, 2006). Farmers recorded data on a provided paper spreadsheet, and were asked to return data for analysis by the 30th June 2020 either electronically via digital photographs or by post using a pre-paid envelope. Farms were removed from the trial if ADG could not be calculated due to poor quality data collection, such as the absence of weighing dates.

After the data collection phase of the trial was completed (30th June 2020), farmers were contacted by telephone and the same management questionnaire was repeated to determine which housing and management practices had been in place during the trial. Where interventions had been implemented part way through the trial period, the management practice that had been in place for the majority of the study time was selected for analytic purposes.

Descriptive and statistical analysis was conducted in R (R Core Team, 2020). An *a priori* sample size calculation was performed, and a target of 60 farms was chosen in order to detect a change in ADG of at least 0.1kg/d based on a SD of 0.13 and power of 80%, with a significance level of 0.05. Incidence risks for mortality and morbidity were calculated as the number of calves having a morbidity or mortality event within the first 90d of life (numerator) out of the calves having a birthweight registered at least 90d prior to the end of the trial (denominator). ADG was estimated for calves where the difference in age between two weights was >40d, to provide an estimation of ADG between birth and weaning. This resulted in four farm level outcomes for analysis, mean ADG between birth and weaning, and the percentage of calves experiencing diarrhoea, pneumonia or dying during the first 90d of life. Incidence risks for morbidity, mortality and ADG rates were calculated at farm level for separate management group groups: dairy heifer calves (DH) and male or beef calves (MB). The number of health plan interventions being implemented both prior to the trial and during the trial period was calculated for both DH and MB groups.

A conventional, simple linear regression model (Kuhn and Johnson, 2013) was created from the data sample using the *lm* function in R (R core team, 2020) with mean ADG at farm level as model outcomes in separate models for dairy heifers and male or beef calves. Intervention group was provided as a fixed effect to evaluate the effect of the calf health plan. The number of interventions implemented were filtered to only include interventions that were in place on >=20% of intervention or control farms and the number of interventions implemented over the trial period was tested as a fixed effect to evaluate the

impact of the number of interventions implemented on calf health and performance. The linear regression models took the form

$$Y_i = \mu + \beta_1 X_{1i} + \epsilon,$$

where Y_i was the mean ADG of the *i*th farm, the fixed effect coefficient β_1 being either intervention group or number of interventions in respective models, μ representing the intercept and ϵ as the random error. The assumed distribution of ϵ was normal, with mean zero and variance θ. The coefficient of determination was calculated utilising 5-fold cross validation (repeated 10 times) within the *caret* (Kuhn, 2018) package using held out (test) data. This was compared with the coefficient of determination calculated from internal model fit (i.e., without any cross-validation), to ensure the internal-calculated coefficient of determination was not dramatically increased compared with cross-validation calculated coefficient of determination (i.e., the model was not over fit). Residual distributions were checked visually to ensure model assumptions were met. To account for the proportional nature of morbidity and mortality outcomes at farm level, beta regression was used (Kieschnick and Mccullough, 2003) within the *betareg* package in R (Cribari-Neto and Zeileis, 2010). The proportion of calves born experiencing either diarrhoea, pneumonia or dying within the first 90d of life was calculated at farm level (0-1) and the following formula (Smithson and Verkuilen, 2006) was used to account for zeros or ones

$$y_i = \frac{y_i(n-1) + 0.5)}{n},$$

where y_i is either diarrhoea, pneumonia, or mortality incidence risk at farm level as a proportion (0-1) of calve born having a case of disease or dying within the first 90d, for the *i*th farm and n is the number of farms. The coefficient of determination for beta regression outcomes was determined using the pseudo r-squared function. As beta regression coefficients are not as intuitive as coefficients from linear regression models, examples were provided by predicting disease rates from beta regression models for farms with the minimum and maximum number of interventions in place, as well as the mean number prior to the trial for all farms, and the mean number at the end of the trial for intervention farms.

5.3. Results

Of the 60 farms enrolled, 14 failed to return any calf records. A total of 3,695 individual calf records were returned from 46 farms. After filtering to remove one farm with unusable records, there were 3,593 calf records from 45 farms (21 CONT farms, 24 INT farms) ranging from 12-263 calves per farm. There were 1,760 calves with two weights recorded >40d apart (955 DH and 805 MB calves) included for ADG calculations, and 1,871 calves

born > 90 d prior to the end of the trial (856 DH and 1,015 MB calves) used as the denominator population for morbidity/mortality calculations. The breed type on all farms were predominantly Holstein Friesian, and calf management practices for control and intervention farms are presented in Figure 5-1.

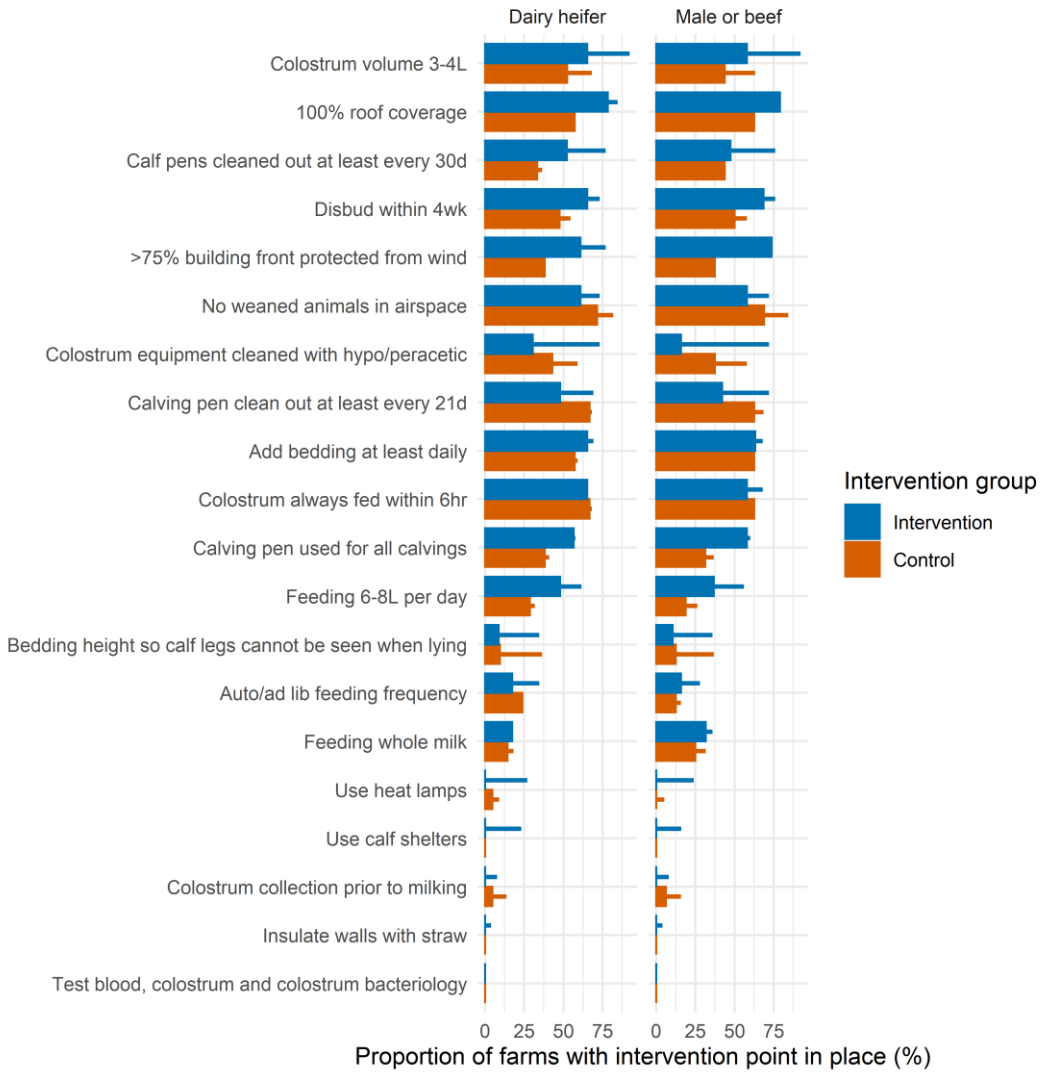


Figure 5-1: The proportion (%) of interventions in place on farms by time and breed type. Proportion of each intervention in place prior to the trial are shown by the bars, with the proportion of interventions in place at the end of the trial are shown by lines. Interventions for dairy heifers are shown left, with interventions for male or beef calves shown right.

The mean ADG for all calves was 0.78kg/d, ranging from 0.33 to 1.13 kg/d, with mean incidence risks of 20.1% (0-96.6%), 16.4% (0-95.2%) and 4.3% (0-18.8%) for diarrhoea, pneumonia, and mortality, respectively. Median (interquartile range, IQR) ADG for all calves was 0.79kg/d (IQR 0.2kg/d), with median incidence risks of 12.8% (IQR 22.3%), 6.7% (IQR 22.2%) and 2.5% (IQR 7.0%) for diarrhoea, pneumonia, and mortality,

respectively. As the questionnaire was conducted with the farmer in person using the herd health toolkit app, farmers answered all required questions. INT farms had a higher number of interventions in place by the end of the trial (Table 5-1). INT farms had a mean of 7.4 interventions in place at the start of the trial, and 9.9 interventions at the end of the trial. CONT farms had a mean of 6.6 interventions in place at the start of the trial, and 7.6 at the end of the trial, despite being given no advice to put any interventions into place. The percentage of CONT and INT farms carrying out recommended interventions at the start and end of the trial are shown in Figure 5-1. Three interventions were identified as being in place on <20% of intervention farms (colostrum collection time being either prior to, during or after milking, insulating concrete walls with straw and routine testing of colostrum or blood to ensure passive transfer) and were removed for the analysis of the association between the number of interventions in place and calf health and production outcomes. ADG was numerically higher in the intervention group for both MB calves and DH calves (Table 5-1). Model results (Table 5-2) indicated that the intervention group was associated with a significant increase in ADG for male and beef calves of 0.12kg/d (95% CI: 0.02-0.22, p=0.03). There were no significant associations between intervention group for mortality or morbidity outcomes (Table 5-2).

The number of interventions in place on a farm, irrespective of intervention group, was significantly associated with several health and production outcomes (Figure 5-2, Table 5-2), regardless of which specific intervention was in place. An increased number of interventions in place was associated with an increase in ADG for DH calves of 0.01kg/d (95% CI: 0-0.03, p=0.04) and MB calves of 0.02kg/d (95% CI: 0.00-0.04, p=0.10) per intervention implemented. The farms with the highest number of interventions in place (15) might expect a ADG of rate of 0.88kg/d and 0.81kg/d for DH and MB calves compared with 0.73kg/d and 0.65kg/d on farms with the lowest number of interventions in place (4) for DH and MB calves respectively. Coefficients for morbidity and mortality models are presented in Table 5-2, however as coefficients for beta regression models are not intuitively interpretable, examples of changes in morbidity and mortality rates associated with the number of interventions in place are presented here. An increased number of interventions in place was associated with a significant decrease in mortality rates for MB calves. For example, the predicted mortality rate on a farm with 7 (mean number of interventions in place for all farms at the start of the trial) interventions in place would be expected to decrease from 7.6% mortality to 5.2% mortality if they increased the number of interventions in place to 10 (mean number of interventions in place for intervention farms at the end of the trial). The farms with the highest number of interventions in place (15) might expect a mortality rate of rate of 2.8% compared with 10.9% for farms with the lowest number of interventions in

place (4). There was a tendency for association between an increased number of interventions in place and a decrease in diarrhoea rates for DH calves, and a farm increasing from 7 to 10 interventions might expect a reduction in diarrhoea incidence risk from 33.1% to 25.2%. The farms with the highest number of interventions in place (15) might expect a diarrhoea rate of 15.1% compared with 42.1% for farms with the lowest number of interventions in place (4). There were no significant associations found between the number of interventions in place and pneumonia rates. Statistical analysis was repeated including the single removed farm to check no significant changes in results occurred; this was found to have no impact.

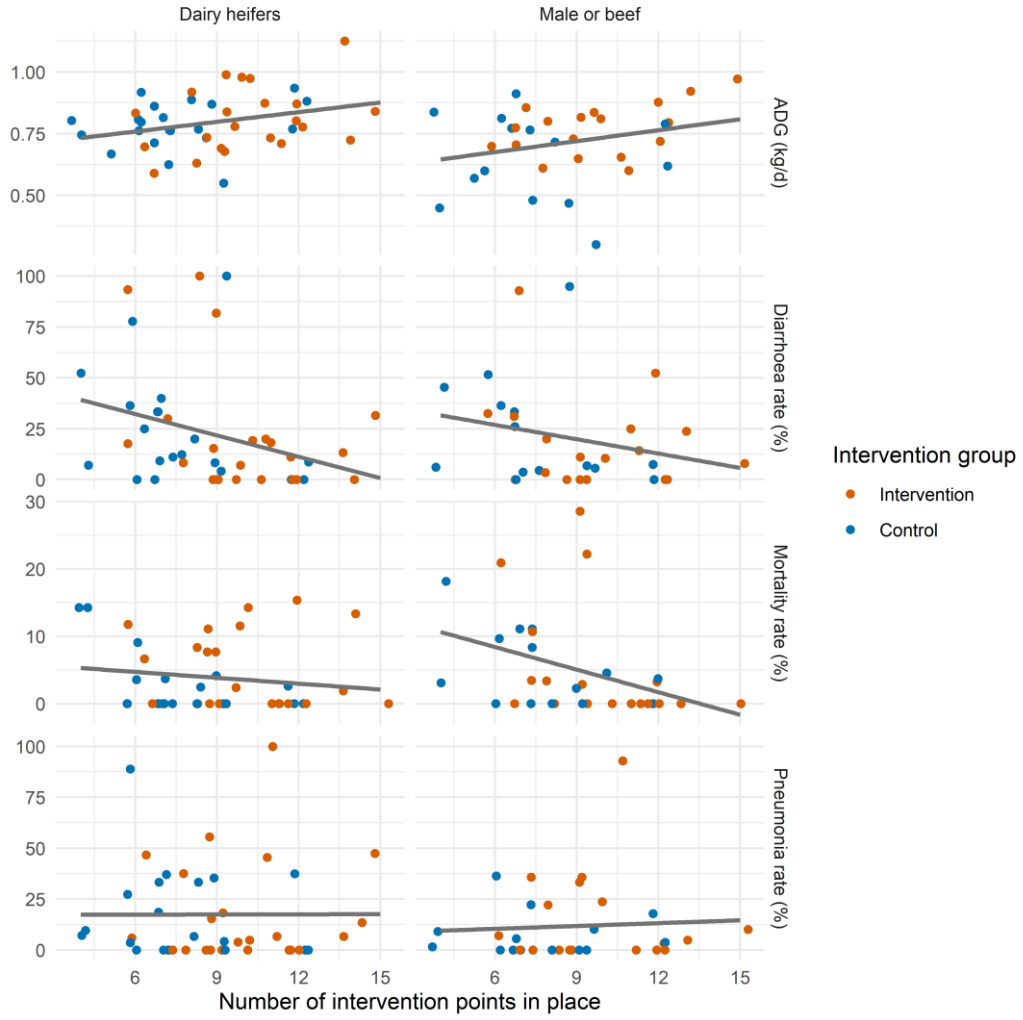


Figure 5-2: Associations between the number of interventions in place and average daily gain, diarrhoea, mortality, and pneumonia rates. Associations between the number of interventions in place and average daily gain, diarrhoea, mortality, and pneumonia rates (panels top to bottom) for dairy heifer calves (left) and male or bull calves (right panels) on intervention and control farms. Each point represents the growth, morbidity or mortality levels for a given farm coloured by their intervention group (intervention or control) with a regression line.

Breed type	Intervention group	ADG denominator farm (calf) numbers	Morbidity/mortality denominator farm (calf) numbers	Number of interventions in place (start)	Number of interventions in place (end)	Change in number of interventions in place	Average daily gain (Kg/d)	Diarrhoea rate (%)	Pneumonia rate (%)	Mortality rate (%)
Dairy heifers	Control	21 (525)	20 (496)	6.68	7.59	0.91	0.78	23.96	17.11	2.71
	Intervention	22 (430)	23 (360)	7.31	10.19	2.88	0.81	20.31	17.71	4.87
Male or beef	Control	14 (273)	14 (455)	6.53	7.58	1.05	0.65	23.01	7.61	5.14
	Intervention	18 (532)	18 (560)	7.40	10.12	2.72	0.77	18.05	14.91	5.30

Table 5-1: Descriptive statistics for dairy heifers and male or beef calves by intervention group. Description of specific interventions in place on farms is provided in Figure 5-1.

		Intervention group (Reference category; Control group)			Number of interventions in place		
Outcome	Breed type	Coefficient (95% CI)	P-value	Coefficient of determination	Coefficient (95% CI)	P-value	Coefficient of determination
Average daily gain (kg/d)	Dairy heifers	0.03 (-0.05-0.1)	0.463	0.13	0.01 (0-0.03)	0.04	0.16
	Male or beef	0.12 (0.02-0.22)	0.025	0.26	0.02 (0-0.04)	0.102	0.27
Mortality (%)	Dairy heifers	0.25 (-0.23-0.74)	0.301	0.03	-0.06 (-0.15-0.03)	0.204	0.03
	Male or beef	-0.15 (-0.72-0.41)	0.595	0.01	-0.14 (-0.24--0.03)	0.015	0.24
Diarrhoea (%)	Dairy heifers	-0.21 (-0.9-0.48)	0.547	0.01	-0.12 (-0.25-0.01)	0.082	0.09
	Male or beef	-0.23 (-0.97-0.51)	0.548	0.02	-0.07 (-0.21-0.07)	0.312	0.04
Pneumonia (%)	Dairy heifers	0 (-0.65-0.66)	0.996	0	-0.01 (-0.13-0.12)	0.884	0
	Male or beef	0.2 (-0.5-0.9)	0.571	0.02	0.01 (-0.12-0.15)	0.857	0

Table 5-2: Results from regression models for both intervention group and number of interventions models. Results from models comparing associations between intervention groups (i.e., intervention and control groups) or the number of interventions in place (i.e., the number of interventions in place during the trial regardless of intervention group) with outcomes average daily gain, mortality, diarrhoea and pneumonia for both dairy heifers and male or beef calves. Coefficients for average daily gain are on the scale kg/d, and coefficients for mortality, diarrhoea and pneumonia are on the logit scale. Coefficient of determination calculated from cross-validation for average daily gain models and pseudo coefficient of determination calculated from internal fit for morbidity and mortality outcomes. P-values deemed significant at $p<0.05$.

5.4. Discussion

This study explored the effect of implementing an evidence-based calf health plan on health and productivity outcomes on dairy farms in Great Britain. The results from this study suggest that the implementation of a calf health plan has the potential to improve ADG for preweaned calves on GB dairy farms, particularly beef cross and dairy bull calves, with each additional intervention in place being associated with improvements in ADG, diarrhoea and mortality rates. With relatively high rates of morbidity and mortality (Table 5-1), and a national focus on improving youngstock health and productivity (CHAWG, 2017), the calf health plan tested in this study represents a potentially useful tool to aid veterinarians and farmers in the implementation of effective management interventions likely to improve the health, welfare, and productivity of preweaned calves on GB dairy farms.

Interventions utilised in this calf health plan were largely based on findings from research described in Chapter 3 identifying factors associated with ADG and were predominantly based around increased feeding levels, limiting the effect of cold stress and improving hygiene. Increased levels of milk feeding and, in particular, the feeding of whole milk, has been previously identified in several studies as being associated with improved health and production outcomes in preweaned calves (Medrano-Galarza *et al.*, 2018; Shivley *et al.*, 2018; Dubrovsky *et al.*, 2019a, 2019b). Despite the added cost of feeding increased levels of milk, it is likely that feeding increased levels of milk would be cost-beneficial in the majority of cases (Dubrovsky et al., 2020), and the feeding of pasteurised whole milk may be an economically viable strategy for dairy farms (Godden et al., 2005). Calves housed in environmental temperatures outside of their thermo-neutral zone are likely to experience heat or cold stress, which could result in direct economic losses through increased calf morbidity and mortality as well as reduced weight gain and performance (Roland et al., 2016) and should, therefore, be minimised in order to maximise calf performance and welfare (Silanikove, 2000). Neonatal calves are prone to heat loss under cold conditions due to their low surface area to mass ratio (Berman, 2003), and are unable to rely on heat production from ruminal fermentation (Collier et al., 1982). In many countries, including Britain, the temperature in calf housing is often below the thermoneutral zone of neonatal calves for a large proportion of the year, with recorded temperatures within calf housing being similar to the recorded ambient temperature as described in Chapter 3. Despite the apparent need for increased environmental temperatures within calf housing, care must be taken when altering buildings for improved heat retention, as housing systems that succeed in providing thermal comfort may predispose other problems such has poor air quality (Roland et al., 2016), and previous studies have suggested that it is preferable to manage

cold stress with the provision of sufficient bedding for nesting rather than the enclosure of pens with solid sides (Lago et al., 2006). The use of external heat sources to increase environmental temperatures has been previously explored, and previous research has suggested that calves show preference for areas under the heat lamp (Borderas et al., 2009) suggesting there is a positive welfare component to the provision of additional heat. These previous studies provide support for the interventions used in this calf health plan relating to the provision of external heat sources and increased bedding provision to allow sufficient nesting. Interventions to improve the housing environment may be beneficial in decreasing the deleterious effects of cold stress on calves in the first few weeks of life. Several housing factors such as the frequent provision of fresh bedding, the use of maternity pen for calving only (Medrano-Galarza et al., 2018), the avoidance of shared airspace with weaned animals and housing under a roof (Maier et al., 2019) have all been previously found to have a positive impact on calf health outcomes. The provision of clean bedding has also been found to be associated with increased ADG in preweaned calves (Quigley et al., 2017).

Whilst we did not identify clear improvements in disease outcomes in farms that implemented the calf health plan, the relatively low number of additional interventions implemented by farms in the intervention group suggests that farmer engagement may play a significant role in the potential efficacy of a health plan. This research demonstrated that increasing the number of interventions on a farm is associated with improvements in ADG, diarrhoea and mortality rates for preweaned calves, but that farmers may be hesitant to implement more than 2-3 interventions over a short time period, limiting the short-term efficacy of the plan. It is likely that small incremental improvements in housing and management over a longer period of time will be beneficial in improving calf health and performance.

Both intervention and control farms reported an increased number of interventions in place at the end of the trial (Table 5-1), suggesting that simply by benchmarking farms, improvements in management practices are likely even where no specific advice has been given. In particular, providing increased levels of bedding and colostrum to calves were relatively commonly improvements made on control farms over the trial period (Figure 5-1), even though no specific advice was provided to do so. This is supported by a previous study which reported an improvement in ADG occurred following the benchmarking of farms, suggesting that the monitoring and benchmarking of calf outcomes might be an effective starting point to improve calf management protocols in practice, irrespective of any additional advice given (Atkinson et al., 2017).

Whilst the efficacy of interventions is clearly important, the practicality of potential interventions and, therefore, farmer willingness to implement a specific intervention is also of importance in the design of a calf health plan. Removal of interventions not commonly implemented has resulted in a more parsimonious plan, which should allow farmers to focus on the smallest number of interventions with the largest potential for positive impact on health and productivity outcomes. The approach taken in this study was based on a risk management tool, consisting of a web app-based interface with 20 simple questions and resulting in a clear list of management factors to focus on. The automated nature of the report means that it is very time efficient to implement and does not require any additional reports to be written as might be typical after a health plan meeting.

Whilst improvements in ADG, diarrhoea and mortality rates have been shown to be associated with an increased number of interventions implemented, there were no significant associations with pneumonia rates. Despite training in the use of a standardised calf health scoring system, there was likely to be significant variations in farmer detection rates for pneumonia, and the sensitivity of respiratory disease detection based on clinical signs was found to be only 27% in a recent meta-analysis (Timsit et al., 2016). Even with a standardised scoring system, the pneumonia rates reported during both the current study and previous study are likely to be influenced by farmer detection capability. The detection of small differences in both disease rates and ADG is also likely to be limited by sample size constraints. High standard deviations in morbidity and mortality rates meant that only relatively large differences could be detected between groups, and a lack of significant associations between intervention group and morbidity or mortality outcomes may again represent insufficient power rather than a true lack of association. With a SD of mean farm ADG of 0.15kg/d and 0.11kg/d and farm numbers of 32 and 43, a difference in ADG of 0.11kg/d and 0.07kg/d would be detectable for MB and DH calves respectively with power of 80% and significance level at 0.05. Therefore, whilst ADG improvements for DH calves on intervention farms did not reach statistical significance by conventional thresholds, this is potentially due to a lack of power and an increased sample size may be required to detect this smaller improvement in ADG. It may also have been challenging to detect differences in growth rates for DH calves, which grew at a relatively high rate in the control group (0.78kg/day). It would be challenging to dramatically increase growth rates far in excess of this relatively high starting figure using the current plan. MB calves, however, had a far lower ADG in the control group (0.65kg/d), and therefore, had far greater potential for improvement in the intervention group. Farm factors not included in the plan are also likely to be influential in ADG rates, in particular the age of weaning due to the non-linear nature of preweaning growth rates (Bazeley et al., 2016). A potential limitation of the modelling

approach is an intervention was considered implemented if it was in place for the majority of the recording period.

Whilst farmers were selected at random for the opportunity to participate in the research project, the voluntary nature of trial enrolment may have resulted in the selection of relatively proactive farmers and, therefore, a degree of bias. Whilst there are some limitations in farm recruitment, as the inclusion within the supermarket group was initially based on convenient milk supply routes any selection bias is likely to be limited and the sample is likely to be relatively representative of GB dairy farms. Some farms were recruited after previous enrolment, again potentially selecting for farmers that were more engaged in the previous study. Farmer recorded mortality (4.28%) was however similar to national mortality levels reported previously (3.87% from Chapter 2) suggesting that this sample is representative of GB, and that farmer recording accuracy was likely to be relatively high. Caution should be taken however, when extrapolating the results from this trial to other countries which may have different health plan requirements to calves on GB dairy farms.

Several interventions identified in the previous study were not implemented as part of the plan, as they were thought to be too difficult to implement over a short period of time, such as increasing the maximum age a calf might reach in their first housing group and increasing the space available per calf. These may be worth further consideration, even if not directly tested in this plan. The coefficient of determination figures provided (Table 5-2) suggest that whilst a reasonable proportion of the variation in health and production outcomes might be accounted for by either the intervention group or the number of interventions in place, there are likely to be other important farm and animal level factors that also bear consideration. Whilst this plan was designed to give relatively rapid improvements to growth rates, it is likely that improvements in ADG are highly multifactorial, and the plan should be utilised as a guide and starting point rather than a holistic health plan.

5.5. Conclusions

This research has demonstrated that implementation of a calf health plan is likely to improve ADG for preweaned MB calves on GB dairy farms, with each additional intervention undertaken being associated with improvements in ADG, diarrhoea and mortality rates for both MB and DH calves. The improvements in management practices made on control farms despite no advice being given suggests that simply monitoring and benchmarking calf health performance might be a useful starting point to improve calf health and performance, with the calf health plan forming a template for further improvements to the housing and management of preweaned calves. The calf health plan tested in this study represents a potentially useful tool to aid veterinarians and farmers in the implementation of effective

management interventions likely to improve the health, welfare, and productivity of preweaned calves on GB dairy farms. The health plan and reporting framework are freely available open access at www.nottingham.ac.uk/herdhealthtoolkit.

Chapter 6 The impact of environmental temperature on average daily gain in preweaned calves; a randomized controlled trial and Bayesian analysis

Environmental temperature appears to be associated with calf mortality rates as discussed in Chapter 2, and growth rates as discussed in Chapter 3. This chapter describes a randomised controlled trial conducted to further explore potential interventions to limit the negative effects of cold stress through the application of calf jackets and/or 1kW heat lamps in a factorial study design. A close derivative of this chapter has been submitted for publication in the Journal of Dairy Science.

6.1. Introduction

Neonatal calves are prone to heat loss in cold conditions due to low surface to mass ratio and poor insulation (Roland et al., 2016), and unlike adult cattle, are unable to generate heat through ruminal fermentation (Collier et al., 1982). Thermoregulatory mechanisms are absent at birth and take up to two months to develop (Piccione et al., 2003; Roland et al., 2016), meaning calves are particularly susceptible to cold stress during the preweaning phase of life.

Previous studies have suggested that environmental temperatures can play an important role in both the growth (Shivley et al., 2018), morbidity (Urie et al., 2018) and mortality rates of preweaned calves as discussed in Chapters 2 and 3. Some studies have suggested that the ability of calves to adapt to colder environments is dependent upon the availability of adequate nutrition, with calves consuming more starter in colder environments than warm, likely compensating for the increased thermal demand (Nonnecke et al., 2009). Reducing heat loss with the liberal provision of deep dry bedding has been advocated (Nordlund, 2008) to reduce the lower critical temperature and eliminate a portion of the additional calorific requirements when housed in cold environments (Lago et al., 2006). In open barns it is been suggested that microenvironments are crucial including being draft free with adequate bedding (Roland et al., 2016). However, there has been limited research into the specific effects of temperature on average daily gain (ADG), or methods of providing additional warmth for calves.

The use of both calf jackets and supplementary heat sources, such as heat lamps, represent potential methods of providing warmer microenvironments for calves, although few studies have been published evaluating their effectiveness. Previous research into calf jackets found no benefit in terms of ADG or immune function (Earley et al., 2004), with subsequent

studies also finding no benefit in ADG (Scoley et al., 2019). Studies exploring the use of heat lamps have found that perinatal calves show a strong preference for heat lamps and may be associated with significant increases in ADG in very early life (Borderas, de Passillé and Rushen, 2009; Bhat *et al.*, 2015). No previous research has examined the potential effects of heat lamp and/or jackets in combination.

A randomized controlled trial (RCT) is a stringent trial design for determining relationships between an intervention and outcome (Sibbald and Roland, 1998). A factorial study design allows for the evaluation of more than one intervention within the same RCT (Montgomery et al., 2003) such as the effect of jacket and/or supplementary heat on ADG. Whilst there are many statistical approaches that would suit the analysis of an RCT, a Bayesian analysis facilitates not only the analysis of the RCT data in isolation, but can also incorporate prior information (Spiegelhalter et al., 2004), such as temperature and ADG data from previous chapters in this thesis.

The aim of this study was to conduct a randomised controlled trial to assess the effects of calf jacket and/or supplementary heat on ADG in preweaned calves. A secondary aim was to evaluate associations between environmental temperature and ADG using a Bayesian approach to incorporate both current, and previous data.

6.2. Materials and methods

A 550-cow dairy farm in the UK was recruited by convenience due to a relatively large herd size, geographical location, and history of accurate data recording.

Calves born after 2nd January 2021 were randomly assigned at birth in a 2x2 factorial design to one of four groups: no jacket, no heat lamp (CON), heat lamp but no jacket (H), jacket but no heat lamp (J) or both heat lamp and jacket (HJ). Randomisation was performed using the *sample* function in R (R core team, 2020) and implemented using a set of sealed envelopes containing group allocation for farm staff to open upon calf enrolment. Blinding of the farm staff to jacket or heat lamp usage was not possible. Calves were weighed using a calf weigh crush (Bateman, Cheddleton UK) and weigh scale (Tru-test EZIWEIGH 5, Datamars Auckland NZ) at birth and 14-35d of age, when beef/bull calves would be sold, and dairy heifer calves were moved to alternate housing. The weigh crush and weigh scale were calibrated every 14d using a known weight. Calf ID, breed, sex and intervention group were recorded alongside birthweight and second weights. Calves weighed <14d between weights were removed from the dataset.

Calves were housed in individual pens (1.4m by 1.0m) with solid sides at 0.9m height. Pens not under heat lamps were arranged side to side in a row of 15 pens in each of the four

quarters of the shed. Pens under heat lamps were arranged in a square consisting of 4 pens with one heat lamp being placed over the centre of all four pens at a height of approximately 1m above the bedding. To limit the effect of any potential within-shed variations, one group of pens both under heat lamp and not under heat lamps were placed in each of 4 quarters of the shed. Four heat lamps (Kiam, Liverpool UK) were set at 1kw and were left on continuously throughout the trial. Data loggers (EL-USB-2, LASCAR electronics, Whiteparish UK) were placed in both heat lamp, and non-heat lamp pens at 30-50cm height within the calf pen and recorded the environmental temperature every 15minutes.

After calving, calves were fed 4L fresh cow's colostrum as soon as possible, and always within 6 hours of birth. A second feed of 2L fresh cows' colostrum was fed approximately 6 hours later. Calves were then fed 3L milk pasteurised at 60^{o}C for 60 minutes using a pasteuriser (Holm and Laue, Westerrönfeld, Germany) harvested from cows vaccinated with Rotavec Corona (MSD Animal Health, Milton Keynes, UK), twice daily until 14d of age. Calves were subsequently fed 4L milk replacer powder (Advanced Superstart 23% CP, Advanced Sourcing, Dunston UK) twice daily at a concentration of 150g/l. Starter pellets (Calf starter 18% CP, ForFarmers, Rougham UK) and fresh water were provided ad-lib to all calves from birth.

6.2.1. Statistical analysis

Statistical analysis was performed in R (R core team, 2020) using the *tidyverse* collection of packages (Wickham et al., 2019). To determine an appropriate sample size, an *a priori* sample size calculation was performed using the *Size.Full* function from the *BDEsize* package (Chung and Lim, 2019). To detect a difference of 0.075kg/d in ADG, 17 calves would be required in each of the four groups at 80% power, a significance level set at $p<0.05$, and a standard deviation of 0.15kg/d, assuming no interactions between treatment groups were present.

Models were fitted on 1,000 bootstrap samples to provide robust estimates of both coefficients and model performance, including 95% bootstrap confidence intervals (BCI) calculated using lower and upper coefficient quantiles of 2.5 and 97.5% across bootstrap samples respectively, using the *tidymodels* collection of packages (Kuhn and Wickham, 2020). The coefficients of determination for fixed effects models were calculated using the *broom* package (Robinson et al., 2021). Bootstrap p-values were calculated as one minus the proportion of coefficient estimates on the majority side of zero. Variables with a bootstrap p-value <0.05 were deemed to have a high probability of having an effect size > or <0. The marginal (variation in pen temperature explained when excluding the random effect of logger ID) and conditional (variation in pen temperature explained when including the

random effect of logger) coefficient of determination for mixed effects models were calculated using the *MuMIn* package (Barton, 2020). Residuals were checked after a model was fitted on the full dataset (i.e., without bootstrap resampling) for near normal distribution (Dohoo et al., 2009).

6.2.2. Associations between treatment group, environmental temperature and ADG

To explore the effect of treatment group (CONT, J, H or HJ) on ADG, a fixed effects linear regression model was fitted, and model parameters estimated using 1,000 bootstrap samples. The model took the form

$$Y_i = \mu + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{1i} X_{2i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \epsilon,$$

where Y_i was the ADG of the *i*th calf. The coefficients were represented by β_1 for jacket usage, β_2 for heat lamp usage, and β_3 as an interaction term between jacket and heat lamp usage. β_4 represent mean outside temperature (°C) and β_5 breed. μ represent the intercept and ϵ as the residual model error. The assumed distribution of ϵ was normal, with mean zero and variance θ.

6.2.3. Bayesian approach to associations between pen temperature and ADG

Associations between environmental temperature and ADG were further explored using a Bayesian approach. Prior information was obtained from previous trial data (Chapter 3) including 484 calves from 16 farms with both ADG calculated from two weigh tape measurements and temperature data for every day of life between these two dates. The impact of temperature on ADG was estimated using a mixed effects model as

$$Y_{ij} = \mu + \beta_1 X_{1ij} + U_j + \epsilon_{ij},$$

where Y_i was the ADG of the *i*th calf on the *j*th farm, β_1 represented the coefficient for the effect of environmental temperature between birth and weaning on ADG for the *i*th calf on the *j*th farm. μ represent the intercept, U_j the random effect of logger and ϵ_{ij} the random error. The assumed distribution of U_j and ϵij was normal, with mean zero and variance θU and $\theta\epsilon$, respectively. The distribution of β1 was used as a prior for a Bayesian model, such that the posterior was the joint probability distribution combining the prior and likelihood and for the outcome ADG (Muth et al., 2018). Markov chain Monte Carlo (MCMC) chains were used, with 3 chains and over 10,000 iterations with the first 1,000 discarded as warm-up using the *rstanarm* package (Goodrich et al., 2020). MCMC chains were visually checked for convergence, and posterior predictive checks over 100 repeats were conducted to ensure distributions of simulated data was analogous to the real data. 95% credible intervals were calculated using the *posterior_interval* function and coefficient of

determination was calculated using the *bayes_R2* function within the *rstanarm* package (Goodrich et al., 2020) based on Gelman et al (2019).

6.2.4. Impact of heat lamp on pen temperature

To explore the effect of heat lamp usage on mean daily pen level temperature a mixed effects model with data logger included as a random effect was built and model parameters estimated using 1,000 bootstrap samples. The model took the form

$$Y_{ij} = \mu + \beta_1 X_{1ij} + \beta_2 X_{2ij} + U_j + \epsilon_{ij},$$

where Y_i was the mean daily temperature of the *i*th day for the *j*th data logger. The coefficient was represented by β_1 for the mean daily outside temperature and β_2 for heat lamp use as a binary categorical variable. μ represent the intercept, U_j the random effect of logger and ϵ_{ij} the residual error. The assumed distribution of U_j and ϵij was normal, with mean zero and variance θU and $\theta \epsilon$, respectively.

The study was conducted following ethical approval from the University of Nottingham Committee for Animal Research and Ethics (3281 201116).

6.3. Results

A total of 112 calves were enrolled with both a birthweight and weaning weight between 2nd January 2021 and 6th April 2021. Ear tag recording errors were made when recording birth weights for 3 calves, and these animals were removed from the dataset. Heat lamp pens were unavailable for 8 calves that were allocated to the heat lamp group, and these animals were removed from the dataset. Twenty-five calves were sold before a second bodyweight measurement had been made and were removed from the dataset. Seventy-nine calves were included in the final dataset, consisting of 37 female calves and 42 male calves. Calf breed was predominantly Aberdeen Angus cross Holstein Friesian (n=66), with some Holstein Friesian (n=8) and Belgian Blue cross Holstein Friesian (n=5).

A total of 57,182 temperature recordings were taken from 20 data loggers across the study period. For analysis of the effect of heat lamp on pen temperature, there were 323 pen-days available in the final dataset and for analysis of associations between treatment group, breed and environmental temperature and ADG, there were 79 calves with >=14d between weights.

Group	Number of calves	Mean ADG (kg/d)	Mean age at second weight (d)	Mean birth weight (kg)	Mean second weight (kg)
No lamp, no jacket	23	0.57	21	46.53	58.09
No lamp, jacket	22	0.57	19	45.38	56.30
Lamp, no jacket	17	0.61	18	45.79	57.02
Lamp and jacket	17	0.64	19	43.15	55.48

Table 6-1: Descriptive results of weights, ages and average daily gain by treatment group.

Calves were weighed with a mean of 19d (14-33d) days between weights and the mean ADG of calves was 0.59kg/d across all calves, with a mean ADG of 0.57kg/d for CON, 0.57kg/d for J, 0.61kg/d for H and 0.64kg/d for HJ groups respectively (Table 6-1, Figure 6-1). The mean pen temperature was 9.19°C and mean daily temperature ranged from -1.59°C to 25.16°C (Figure 6-2). The mean temperature of pens with heat lamps was 11.98°C (range 3.67°C to 25.16°C) compared with a mean pen temperature of pens without heat lamps of 7.11°C (range -1.59°C to 19.20°C). Therefore, the mean daily temperature was 4.9°C higher in pens with heat lamps than those with no heat lamps. There was minimal difference between ambient shed and outside temperatures (0.04°C), consistent with previous findings discussed in Chapter 3.

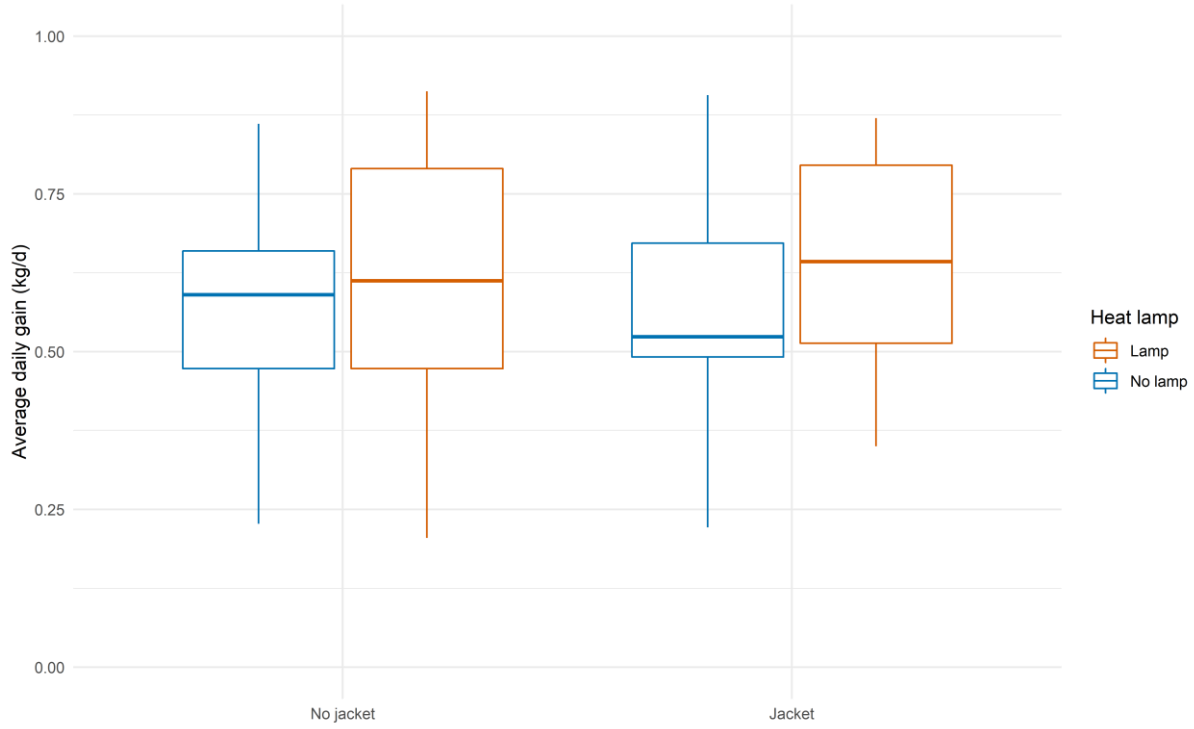


Figure 6-1: Boxplots of daily gain (kg/d) by intervention group.

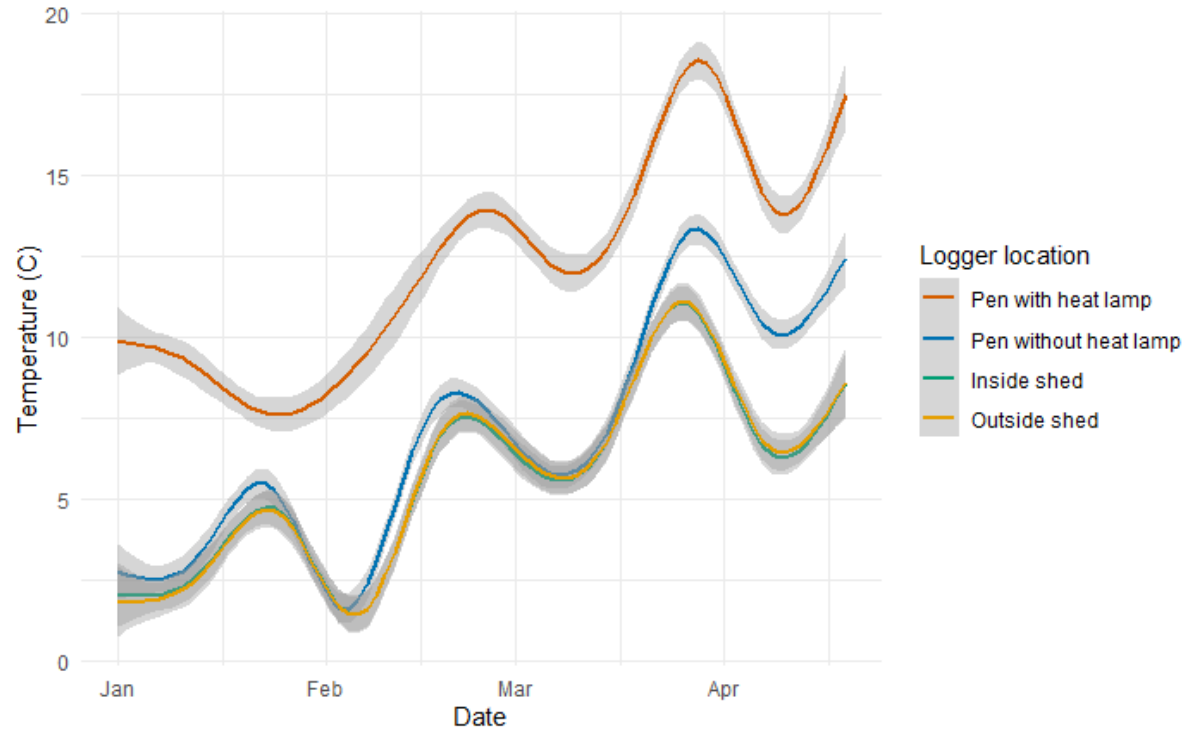


Figure 6-2: Mean temperature (°C) over time by logger location.

6.3.1. Associations between treatment group, environmental temperature and ADG

Results from the final mixed effects model demonstrated that calves housed under heat lamps had a significantly increased ADG (0.09kg/d, 95% BCI -0.02-0.20kg/d, bootstrap p-value=0.047, Table 6-2, Figure 6-3) than those without heat lamps. No effect of jacket or any interactions between jacket and heat lamp were found. An association between increased mean environmental temperature and increased ADG was identified, with a 1°C increase in building temperature being associated with a 0.03kg/d increase in ADG (95% BCI 0.01-0.04kg/d, bootstrap p-value =0.002). A significant association between calf breed was also found, with Belgian Blue cross calves growing faster (0.16kg/d, 95% BCI -0.03-0.30kg/d) and Holstein Friesian calves growing slower (-0.16g/d, 95% BCI -0.27--0.06kg/d) than the reference breed Angus cross.

Term	Mean coefficient (95% BCI)	Bootstrap P-value
(Intercept)	0.39 (0.29-0.50)	
Breed Blue (ref Angus)	0.16 (-0.03-0.30)	0.036
Breed Holstein (ref Angus)	-0.16 (-0.27--0.06)	<0.001
Jacket use	0.04 (-0.06-0.14)	0.231
Lamp use	0.09 (-0.02-0.20)	0.047
Lamp: Jacket interaction	-0.05 (-0.20-0.11)	0.276
Environmental temperature (°C)	0.03 (0.01-0.04)	0.002

Table 6-2: Linear regression model results of associations between treatment group, breed and mean environmental temperature and average daily gain (kg/d) over 1,000 bootstrap repeats. Mean coefficient of determination was 0.27 (95% BCI 0.13-0.44). Coefficient estimations for categorical variables are associated with the change in average daily gain (kg/d) associated with the variable compared with the reference category being "No use" for lamp and jacket use and "Angus cross" for breed.

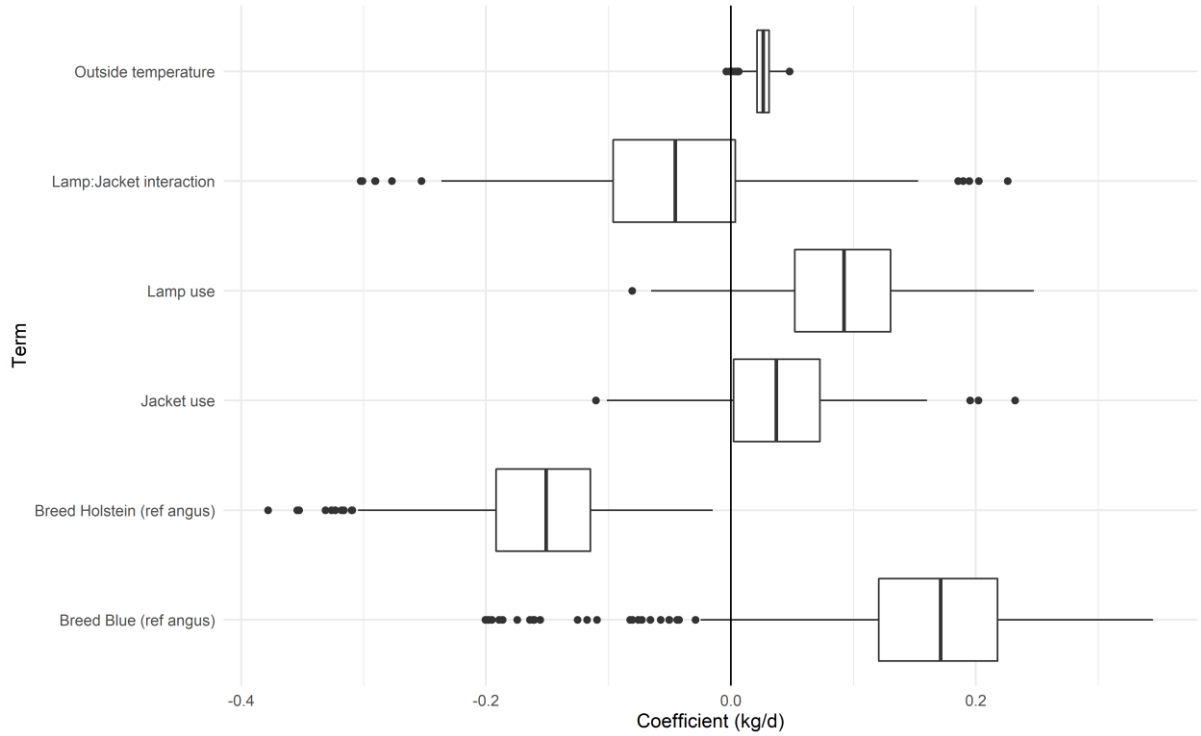


Figure 6-3: Coefficient distributions for associations with average daily gain. Coefficient estimations for temperature represent the change in average daily gain (kg/d) associated with a 1°C change in outside environmental temperature, and coefficient estimations for categorical variables are associated with the change in average daily gain (kg/d) associated with the variable compared with the reference category being "No use" for lamp and jacket use and "Angus cross" for breed.

6.3.2. Bayesian approach to associations between pen temperature and ADG

Visual description of associations between pen temperature and growth rates for the 16 farms supplying data for the prior distribution, and the current study farm are shown in Figure 6-4.

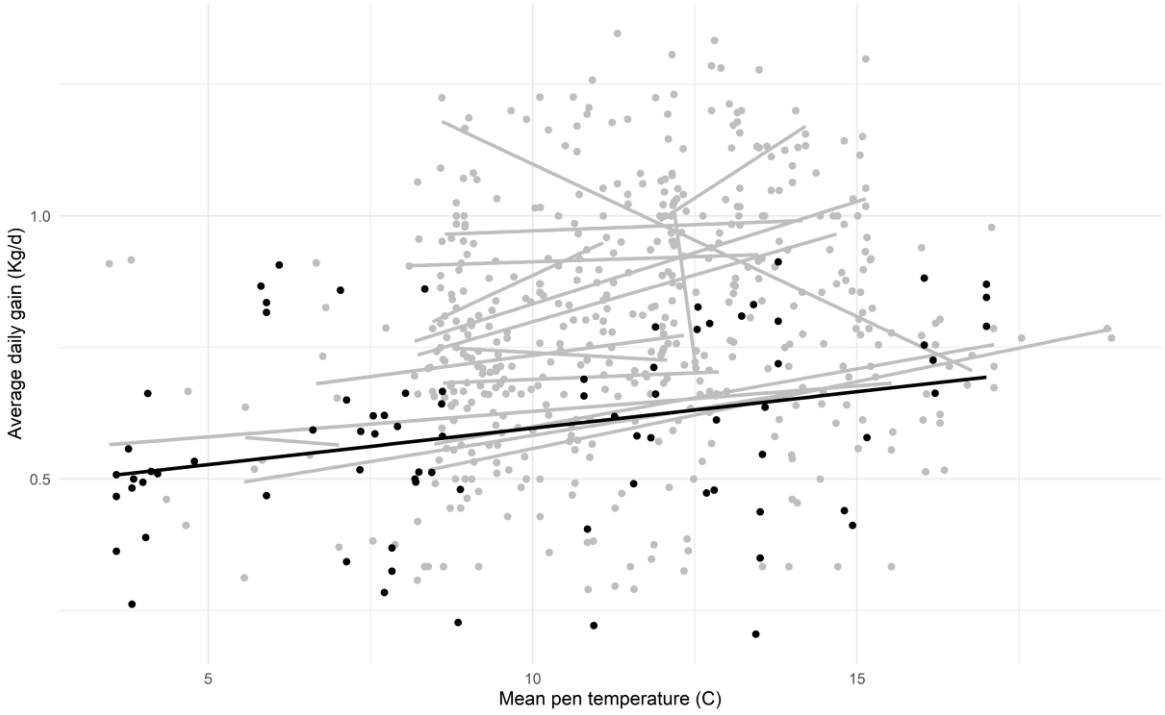


Figure 6-4: Descriptive summary of associations between mean pen temperature (°C) and average daily gain (kg/d). Data is coloured for 484 calves from 16 farms on the previous trial (grey) and the 79 calves from the single farm on the current trial (black). Points represent individual calves and lines represent a linear regression line for each farm.

The prior estimated from the mixed effects model using data from the previous trial was normally distributed with mean 0.015kg/d and 95% CI 0.008-0.023kg/d (increase in ADG per 1°C increase). Likelihood estimates based on weakly informative priors were 0.014kg/d (95% CI 0.004-0.024 kg/d) per 1°C increase. Posterior estimates were 0.014kg/d (95% CI 0.009-0.021 kg/d) per 1°C increase, and the median Bayesian coefficient of determination was 0.11 (95% CI: 0.04-0.20). The distributions of prior, likelihood and posterior estimates are shown in Figure 6-5.

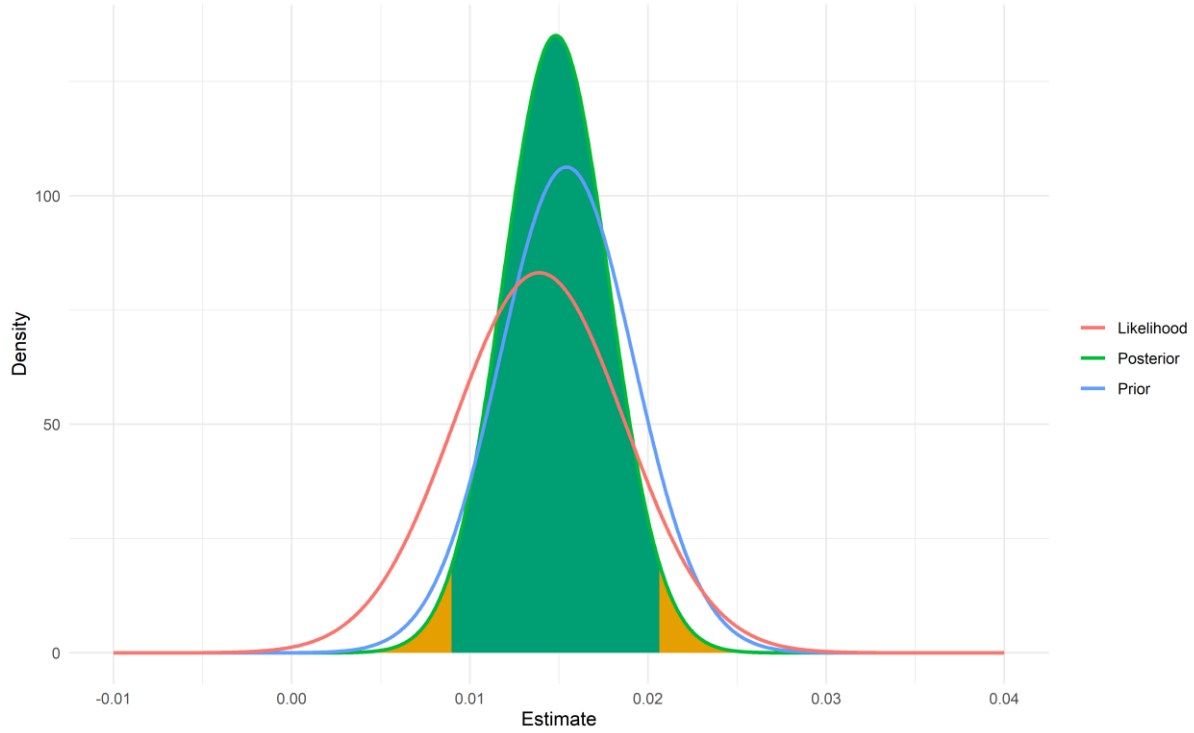


Figure 6-5: Descriptive presentation of prior, likelihood and posterior estimates of associations between a 1°C increase in pen temperature and average daily gain (kg/d). The shaded area represents the 95% credible intervals for the posterior distributions.

6.3.3. Impact of heat lamp on pen temperature

Outside environmental temperature and heat lamp usage were both associated with pen temperature, with a 1°C increase in outside temperature being associated with a 0.99°C increase in pen temperature (95% BCI 0.93-1.05°C) and heat lamp usage being associated with a pen temperature increase of 4.73C (95% BCI 4.43-5.01°C). Bootstrapped mean marginal coefficient of determination was 80% (95% BCI 0.75-0.83) with a conditional coefficient of determination of 88% (95% BCI 0.85-0.91).

6.4. Discussion

The results of this randomised controlled trial suggest that 1kW heat lamps have a significant positive impact on ADG in preweaned calves, and calf jackets were not significantly associated with ADG. Results also suggest that average daily gain in calves is significantly associated with environmental temperature. The positive effect of environmental temperature on ADG identified in the current trial was further reinforced when prior information from previous research was integrated through a Bayesian modelling approach. This represents an important factor for consideration in the improvement of productivity of calves on GB dairy farms.

Increasing temperatures in calf buildings without supplementary heat input is likely to be extremely challenging in GB, and the current study data suggest that without such heat sources, there are minimal differences between the internal and external temperature of calf buildings, as found in both the current study and previous studies discussed in Chapter 3. The use of heat lamps was associated with an increase in pen temperature of ~5°C, and calves housed under heat lamps in the current study grew at a higher rate than those not housed under a heat lamp. 1kW heat lamps, as used in this study, could be used to improve calf growth rates in GB and would be expected to increase in growth rates in early life by ~90g/d when used in colder months.

Alongside improvements in productivity, previous studies have suggested that calves tend to show a preference for heated areas, suggesting there may be a positive welfare aspect to the use of heat lamps (Borderas et al., 2009). Whilst heat lamps may represent a useful adjunct to improve the ADG of preweaned calves, there are both economic and safety considerations. A 1kWh heater uses 504kWh per 21d period. Based on a mean electricity cost of £0.13 ($0.19) per kWh in 2020 (UK Government, 2021), the heater would cost £67.74 ($94.27) per 21d to run if left on constantly. This cost would be split amongst 4 calves in the current study, at a cost of £16.93 ($23.56) per calf. It may be possible to share a heat lamp between a higher number of calves if in a loose housed pen, however, there may be a disease risk if large numbers of calves are crowding underneath a heat lamp which bears consideration. Further cost-benefit analysis would be necessary to determine if the running cost is likely to be a worthwhile investment for the potential increases in ADG. The carbon cost of running heat lamps is also worth consideration, although renewable energy generation may mitigate this depending on the electricity source. It is also worth considering the safety aspects of using heaters within livestock buildings. The heat lamps in the current study were supported from steel girders with steel cable to ameliorate the risk of heat lamps being knocked into straw bedding which could have disastrous consequences.

The type of heater is also likely to be important, and preliminary investigations suggested a more typical 250W livestock heat lamp used for lambs or poultry might only provide a ~1°C increase compared with the ~5°C increase seen with a 1kW heater suspended above a calf pen at 1m above bedding. It is also worth noting that the data loggers used in this trial measured environmental temperature and did not measure direct radiant heat as might be measured with a black globe temperature probe. Increases in temperature under heat lamps compared with no-heat lamps as measured by a black globe probe, however, were comparable to those recorded from the data loggers during preliminary investigations. There is a trade-off in calf housing design between maintaining a warm environment for the calves, and the provision of fresh air crucial in reducing the risk of respiratory disease (Nordlund,

2008), and whilst pen temperature appears to be important in calf growth, the pursuit of a warm environment at the expense of adequate ventilation may increase the risk of respiratory disease outbreaks.

As reported in previous studies (Earley et al., 2004; Scoley et al., 2019), jacket usage was not significantly associated with ADG. This finding is consistent with results from previous studies, and despite their relatively common presence on farms, jackets by themselves are unlikely to result in substantial improvements in ADG.

The RCT component of the current study is limited to a single UK farm, and caution should be taken with the extrapolation of these results to the wider industry, and particularly to different countries and climates. Calves in this RCT were individually housed, and although previous reports have suggested that calves being physically active might increase heat production and therefore cold resistance (Roland et al., 2016), the housing of animals in relatively small pens might hinder the ability of these animals to mitigate cold stress through movement. Similarly, other behavioural adaptations to cold such as crowing or huddling (Roland et al., 2016) would not be possible in individual housing. One limitation of the current study is the relatively high variability in ADG, which was higher than anticipated when performing the sample size calculations. Increased variability in weights at calf rather than farm level during early life has been reported previously (Archer, 2021), however, due to the commercial practices of the farm in the current study, it was not possible to retain calves for longer periods of time to reduce the variability in ADG and thereby increase the likelihood of finding a significant result. Further work should explore the effect of heat lamps and jackets over a longer period of time to reduce the variability between calves. Analysis of the study population (Table 6-1), show more calves did not receive lamps than did receive lamps, and it is possible that farm staff might not have recorded some of the cases when heat lamp were available due to limited numbers, and instead of being removed from the trial they may have been allocated to a non-heat lamp group instead. Analysis of the effect of treatment group on morbidity and mortality levels would be interesting in future studies, however, the sample size required for a binary, relatively low incidence outcome was far in excess of the available calf numbers in this instance.

A Bayesian approach allowed the incorporation of both information excluding the trial data (*prior*) and current data (*likelihood*) to form a final estimate (*posterior*) of an association between a variable and a given outcome (Spiegelhalter et al., 2004). The accuracy of an estimate of associations between environmental temperature and ADG is likely to be improved when incorporating both the data from the current trial and data from previous research discussed in Chapter 3 in a Bayesian framework. Growth rates in early life are

likely to be multifactorial and the relatively low Bayesian R^2 for associations between growth rates and environmental temperatures (0.11) suggest there are other factors associated with ADG in addition to the impact of environmental temperatures demonstrated in the current study.

6.5. Conclusions

Results from this randomised controlled trial demonstrate that 1kW heat lamps have a significant positive impact on the ADG of preweaned calves on GB dairy farms. Environmental temperature was positively associated with ADG in calves, which was reinforced when prior information from previous research was integrated through a Bayesian modelling approach. This represents an important factor for consideration in the improvement of productivity of calves on GB dairy farms.

Chapter 7 Discussion and Conclusions

7.1. Discussion

This research utilises a range of statistical learning techniques to identify factors associated with calf performance, which have ultimately been tested in a randomised controlled trial. To provide farmers and veterinarians with access to the calf health findings of the thesis in interactive form, the University of Nottingham Herd Health Toolkit (www.nottingham.ac.uk/herdhealthtoolkit) was created including tools relating to the management of colostrum, prediction of mortality rates and ultimately a bespoke calf health plan based on user inputs. The toolkit represents a set of tools that are already being utilised by both veterinarians and farmers to improve the health and welfare of cattle, and at the time of writing, the toolkit has been utilised over 2,500 times since its launch in September 2019.

As of 2020, there are currently 2.65 million dairy cows in the UK, and an additional 20.5 million dairy cows within the EU (AHDB, 2021), and as described in Chapter 2, there are around 2.7 million calves born each year in GB. Given the relatively high rates of morbidity and mortality reported both in this thesis and previously (Johnson et al., 2017), there are clearly a large number of animals that would benefit from improvements in management factors related to health and performance outcomes, both in GB and internationally.

Calf survival has been identified as a priority by national bodies (CHAWG, 2018, 2017), and it is crucial that patterns of calf mortality at a national level are routinely analysed and subsequently presented to stakeholders in order to inform resource allocation for both knowledge exchange and future research. The benchmark of calf mortality rates reported in Chapter 2 is the first study published reporting mortality rates of preweaned calves in GB across breed types and sexes. The importance of continued monitoring of national data for temporal trends is highlighted by the discovery of an increasing trend of male dairy calves being sent for slaughter by 3 months of age that had not been previously reported, and there are a range of social and economic factors likely at play that bear consideration for future research. Alongside the identification of temporal trends over time, this national data can also be used to identify factors associated with mortality rates, which could be used to better advise farmers to make evidence-based management changes. This research showed that environmental temperature, time of year, sex, and breed type were associated with mortality rate. At a national level, the mortality rates of both dairy and non-dairy calves would be <2% in optimum environmental conditions, i.e., female calves, born in February, with a mean monthly environmental temperature of around 17°C, a significant decrease from the ~9% mortality rate experienced by male dairy calves born in January. It would seem unlikely that

the breed type or sex of preweaned animals are directly responsible for such large variations in mortality rates, whereas the environmental and management practices for certain groups of calves is more likely to have a significant influence on mortality rates. From the data presented in Chapter 2, it appears that consistently low mortality rates are achievable for a certain calf demographic given specific environmental conditions, which suggests that by altering housing and management practices, improvements in calf mortality rates at a national level across all calf types could be achievable.

The patterns of human mortality presented in Chapter 2 were remarkably similar to those for calves across the same time period, showing a marked increase in mortality rates across winter months, and even similar differences between males and females. Links between temperature and human mortality rates in England and Wales have been previously discussed (Brown et al., 2010), and causes of excess winter mortality in humans is largely driven by respiratory diseases (ONS, 2018). The associations of both season and environmental temperature with mortality rates in calves within this study are strengthened by the similar findings within the human population and suggest an interesting area for further study. Whilst there is certainly the potential to reduce calf mortality levels as a whole, the seasonal patterns identified within both the human and calf population are suggestive that there may be an intrinsically higher risk of mortality over the winter period, and it is possible that seasonal patterns may remain, even if overall mortality levels are reduced.

The most important housing and management factors from Chapter 3 were incorporated into an evidence-based calf health plan, implemented in a randomised controlled trial reported in Chapter 5. The key management factors included within the calf health plan are largely focussed on good nutrition, hygiene, and environmental conditions, and often represent examples of basic animal husbandry. It was relatively common, however, that these management factors were not implemented on the farms visited during this study, and it is possible that the presentation through a formal evidence-based plan might encourage implementation. Benchmarking calf performance itself might also go some way to improving calf management factors. This is evidenced in part by the findings from Chapter 5 that control farms improved management practices over time despite receiving no advice to improve calf management factors, suggesting that farmers might be aware of some management factors they might already need to improve, and may be more incentivised to improve calf management if calf outcomes were monitored and reported more frequently.

Whilst there are many nuances of calf management that can be discussed, keeping calves warm, clean, and dry whilst ensuring they receive large volumes of liquid milk is an

excellent starting point for optimal preweaning calf management. One important factor that has received very little attention previously is environmental temperature, which was identified as being negatively associated with mortality rates in Chapter 2, and positively associated with growth rates in Chapter 3 and Chapter 6. From this research, it is likely that environmental temperature is an important factor associated with growth and mortality in preweaned calves. It was shown in Chapter 3 that the temperature within calf buildings is very similar to ambient environmental conditions, and it would appear that building design is unlikely to play a major role in maintaining higher environmental temperatures during the winter months. Building design is clearly of great importance, however, in providing shelter from rain/wind whilst being easy to access and clean. There are many potential options for increasing temperatures over winter for preweaned calves and improving the nesting ability by providing high levels of deep dry bedding or providing microclimates of shelter in otherwise exposed conditions are likely to help to at least some degree. The provision of supplemental heat comes at a higher economic cost but has been demonstrated in Chapter 6 to be effective in improving growth rates of preweaned calves in colder conditions. The type of heater is also likely to be important, and preliminary investigations suggested a more typical 250W livestock heat lamp used for lambs or poultry might only provide a ~1°C increase compared with the ~5°C increase seen with a 1kW heater. Jacket usage was not significantly associated with calf growth rates, which is supported by previous studies (Earley et al., 2004; Scoley et al., 2019), but despite the lack of empirical evidence as to any benefits, remain relatively common on British farms. Despite a lack of evidence as to benefits in terms of productivity, there may be some welfare benefit to calves wearing jackets, and they should not be discarded based on this research alone.

The importance of colostrum management in calf health has been reinforced within this thesis. In support of previous studies, colostrum collected directly from the cow's teat was unlikely to have high total bacterial, or coliform counts compared with samples collected from collection or feeding equipment, suggesting that the risk for contamination of colostrum comes largely from improperly cleaned feeding equipment. This is supported by the large numbers of farmers using either cold parlour wash or water to wash out collection or feeding equipment, which is extremely unlikely to be sufficient in terms of reducing bacterial load. Previous research has suggested thresholds for bacterial contamination as being at >100,000 cfu/ml and >10,000 cfu/ml for total bacterial counts and coliform counts respectively (McGuirk and Collins, 2004). Given that there is a negative threshold between coliform counts and serum IgG (Godden et al., 2012), it would seem preferable that the target for coliforms was simply as low as possible (i.e., 0 cfu/ml). A target of zero coliforms would appear to be achievable, and as reported in Chapter 4, 41% of samples from feeding

equipment had a 0 cfu/ml coliform count. The world health organisation recommended targets for human drinking water is zero coliforms per 100ml (WHO, 2017), and given that it is clearly achievable within colostrum, there would appear no reason to aim for anything else.

A potential route for colostrum contamination not previously reported is the higher levels of bacterial contamination found when collected through robot milking machines. Through discussions with several robot milking farmers, it was found that the default settings of robots were to not perform a machine wash or perform a pre-milking teat cleaning protocol prior to colostrum collection. This is potentially a significant omission in default robot settings, particularly given that these protocols are conducted prior to regular (non-colostrum) milking. Whilst these settings can be altered on a farm it is likely that these settings are infrequently altered by farmers. It is therefore important that veterinarians on robotically milked dairy farms test colostrum samples for bacterial levels and investigate default colostrum collection settings to ensure that the same cleaning protocols utilised prior to milk collection are also performed prior to colostrum collection.

The provision of high-quality veterinary advice for calf health can be time consuming, and whilst there were some associations between increased veterinary input and calf performance reported in Chapter 3, it can be challenging for veterinarians to find time to create bespoke benchmarking reports for a group of farms. The automated report writing potential of the calf health toolkit discussed in Chapter 5 represents a time efficient method of providing a written report to the farmer, which is bespoke to their needs. The automated nature of the report writing code means that farmers receive both a benchmark report of their current management practices, and a list of recommendations most likely to be effective on their farm (*Appendix ii*). The ability to produce multiple calf health reports at the click of a button means that veterinarians are now able to regularly provide benchmarking reports and have more time available for discussing the practicalities of implementing improvements in management with the farmers rather than writing reports. The automated report framework has been provided for use by veterinarians free of charge as part of the herd health toolkit and would have great potential in many areas of herd health management.

A number of statistical learning techniques within the field of stability selection were developed in parallel to this thesis, such as the development of an empiric threshold for stability selection, ultimately resulting in the creation of the *stabiliser* R package (Hyde et al., 2021) to allow these techniques to be utilised by the wider research community. The statistical challenges faced within this thesis precipitated the development of several new techniques, and in particular dealing with the p>>n problem highlighted the potential failures

of conventional modelling approaches. The use of regularised regression in combination with stability selection techniques provides a robust alternative to conventional methodology, and the development of the *stabiliser* package means that these techniques can be easily applied in future research.

7.1.1. Limitations

Whilst every attempt was made to standardise the collection of morbidity data, the recording of disease by farmers was relatively poor, as has been reported in previous studies (Johnson et al., 2017; Sivula et al., 1996; Virtala et al., 1996), in particular, the detection of respiratory disease (Timsit et al., 2016). The creation of the calf plan was largely based on the findings from Chapter 3, and whilst ADG recording appeared to be relatively accurate, disease recording data was frequently missing or poorly recorded. Farmers often commented that the disease recording was less than accurate, and it was also not uncommon for farmers to report zero pneumonia cases, despite animals being heard to be coughing upon farm visits. A major limitation of this study was the poor recording of pneumonia cases in Chapter 3, where analysis of pneumonia rates was only possible for 15 farms out of 60 initially recruited. Analysis to identify factors associated with pneumonia rates were limited firstly by the small sample size, but also by the likely high levels of inaccuracy in the 15 farms where pneumonia data was present. A dataset with a small number of observations and relatively high random error means identification of causal variables from a large number of variables is relatively challenging, as demonstrated by the low numbers of variables identified as being stable when analysing factors associated with pneumonia in Chapter 3. Diagnosis of pneumonia by farmers is likely to have relatively poor sensitivity and specificity, and whilst it might prove prohibitively costly to implement, future studies might either utilise veterinary diagnosis, which has been suggested to be more accurate/sensitive (Virtala et al., 1996), or through more objective scoring methods of lung damage such as thoracic ultrasound (Buczinski et al., 2014).

The generalisability of any study is of great importance. As the dataset used in Chapter 2 was a national database it is clearly generalisable to GB, however, extrapolation of these results to other countries, particularly with different climates, should be made with caution. There may be differences between the farms randomly selected from a supermarket supply group and the general dairy farm population in GB for Chapters 3-5 however, since farms were initially selected for inclusion within the supermarket group based on convenient milk tanker routes, any initial selection bias is likely to be limited. There is also a potential selection bias implicit within any study requiring voluntary involvement, and it is possible that more proactive or agreeable farmers might be selected for inclusion within the study

population. The recruitment of a single farm for Chapter 6 was based on convenience, size, and previous quality of data recording, and may not be representative of GB dairy farms. Whilst Chapter 2 involved mortality data across all months for multiple years, the data for Chapters 3-6 were largely between December and June, and findings may vary during different time periods or environmental conditions.

A number of statistical challenges were encountered during the development of this thesis and highlighted the limitations of conventional approaches in the robust identification of truly causal variables from a relatively high dimensional dataset. The use of statistical learning techniques such as regularised regression in combination with stability selection were a largely successful solution utilised throughout the thesis, although further improvements in these techniques were identified such as the development of an empiric stability selection threshold and the use of multiple model triangulation within a stability selection threshold. Whilst these techniques were successfully developed and tested on simulated datasets towards the end of development of the thesis, they have not been implemented in the analysis or development of the calf health plan. The use of statistical learning techniques and stability selection in combination with the use of an empiric stability selection threshold and the use of multiple model triangulation would be worthwhile in future studies attempting to identify causal variables from within a high dimensional dataset within calf health studies but would also be applicable to the wider fields of epidemiological research.

Whilst there are a number of potential limitations that bear consideration, the studies reported within this thesis were designed to ameliorate these limitations where possible. The use of a randomised controlled trial design was utilised in both Chapter 5 and Chapter 6, to specifically test the effects of the calf health plan, and supplementary heat respectively, and represent the most rigorous method of determining a cause-effect relationship (Sibbald and Roland, 1998). Whilst the identification of potentially causal variables reported within research must always be taken with a degree of scrutiny, the positive associations reported from randomised controlled trials reported in Chapters 5 and 6 provide further robust evidence that associations truly exist between the management factors tested and calf health and performance outcomes.

7.2. Future studies

Whilst colostrum bacteriology was explored within this thesis, there was not the opportunity to explore milk feeding bacteriology levels. Given that a large proportion of the bacterial contamination originated from colostrum collection and feeding equipment, it would not be unreasonable to assume the same was true for milk feeding, and by repeating the colostrum

bacteriology study from Chapter 4 for milk or milk replacer it would be possible to determine firstly, whether animals are likely to be routinely ingesting high levels of bacteria via milk feeding, as well as identify potential factors associated with high levels of contamination.

The association between environmental temperature and both growth and mortality reported in Chapters 2 and 6 appears relatively clear, however, the cost-effectiveness of potential interventions in terms of both finance and greenhouse gas emissions has not been explored within this thesis. Cost-benefit is an important consideration prior to the implementation of any management change on farms, and a full cost-benefit analysis of providing 1kW heat lamps on the growth rates and reductions in mortality rates would be an interesting area for further work.

There remains a great deal of knowledge transfer required around the feeding of calves. Calves fed only 6L a day have been reported to show chronic signs of hunger (Rosenberger *et al.*, 2017), however, only 8 of the 30 farms reported in Chapter 3 were feeding greater than 6L/d of milk or milk replacer. Feeding higher quantities of milk was found to be associated with improved growth rates in this research but was also associated with a reduction in the incidence of diarrhoea. The feeding of whole milk, as opposed to milk replacer, was found to be even more important in the reduction of diarrhoea cases, and both findings suggest that feeding practices replicating a calf suckling from a dam (large volumes of whole milk) is likely to be a reasonable aim. There is a growing body of evidence that suggests calves are less susceptible to succumbing to diarrhoea when nursing with a cow, and whilst concerns exist around the potential for transmission of diseases such as *Mycobacterium avium subspecies paratuberculosis* to calves via whole milk, recent literature reviewing cow-calf separation suggest there is minimal evidence to support these concerns (Beaver et al., 2019). Further studies might investigate whether whole milk might be of benefit in improving calf health and production outcomes compared with milk replacer powder whilst considering the potential risk of other transmissible diseases from the adult herd.

7.3. Conclusions

7.3.1. Chapter 2

Despite the legal requirement to record the births and deaths of all cattle in GB, there has been no previous estimate as to the rate of mortality in preweaned calves in GB. The use of the GB national cattle register provided a resource from which to calculate mortality rates for various calf demographics and would provide valuable insights to the industry if

regularly reported in the future. Alongside an initial benchmark for calf mortality in GB, multivariate adaptive regression spline models were utilised to explore factors associated with calf mortality rates, and it was found that environmental conditions are likely to play a significant role, with increased environmental temperatures being associated with lower mortality rates in colder months. This study suggests that farmers might decrease mortality rates by improving the environmental conditions for preweaned calves and developing practical solutions increase environmental temperatures for calves in colder conditions should be a future aim of the cattle industry. The findings of this research have been made accessible within the University of Nottingham Herd Health Toolkit, where mortality rates can be predicted at a national level for given environmental conditions.

7.3.2. Chapter 3

To determine which management factors were associated with calf performance on GB dairy farms, 60 dairy farms were visited across GB. Detailed management data were collected, including feeding, colostrum, building and general management information, as well as recording environmental conditions across the trial period. Elastic net models were utilised alongside stability selection to identify factors with the potential for major improvements (large effect size) on the majority of farms (high stability) on farmer recorded performance outcomes. It was found that increasing the maximum preweaned age within the first housing group (limiting group changes), increasing both milk feeding volume and environmental temperature, ensuring regular cleaning out of both group housing pens calving pens and ensuring rapid feeding of colostrum were found to be particularly important in improving ADG at farm level.

7.3.3. Chapter 4

Colostrum samples were submitted by 59 farms for bacteriology testing, and alongside providing an initial benchmark of bacteriology levels found in colostrum on GB dairy farms, factors associated with high levels of bacterial contamination were also explored, using mixed effects models alongside stability selection. Whilst some farms were able to achieve extremely low levels of bacterial contamination, the study indicated that over one third of samples collected from buckets or feeding equipment were over contamination thresholds and represent a significant risk for both ingestion of pathogens and increasing the risk of passive transfer of immunity. Colostrum samples collected directly from the cow's teat had lower levels of bacterial contamination, suggesting that microbiological contamination is likely to occur from improperly cleaned equipment rather than directly from the cow, and the colostrum from robot milking herds were identified as being at particular risk of contamination.

The findings from this study resulted in several key recommendations including the cleaning of colostrum collection and feeding equipment after every use with hot water as opposed to cold water, and hypochlorite or peracetic acid as opposed to water or parlour wash. Cows' teats should be prepared with a pre-milking teat disinfectant and wiped with a clean, dry paper towel prior to colostrum collection, and colostrum should be pasteurised where possible. Veterinarians and farmers should consider performing colostrum bacteriology as a routine practice and should ensure protocols include appropriate cleaning including the use of scalding hot water, and either hypochlorite or peracetic acid. The findings of this research have been made accessible within the University of Nottingham Herd Health Toolkit, where farmers and veterinarians can receive bespoke colostrum hygiene recommendations based on their current colostrum management practices.

7.3.4. Chapter 5

Building on the exploratory studies reported in Chapters 2-4, a calf health plan was created based on the research evidence, largely focussing on hygiene, feeding and environmental management. This plan was tested as an intervention compared with no plan in a randomised controlled trial through the enrolment of 60 farms across GB. This study demonstrated that the implementation of this calf health plan is likely to improve growth rates for preweaned calves, particularly male or beef calves. The implementation of additional interventions on farms was also associated with improvements in growth rates, diarrhoea, and mortality rates. This calf health plan represents a potentially useful tool for veterinarians and farmers in implementing effective management interventions aiming to improve the health, welfare, and productivity of preweaned calves on GB dairy farms. Veterinarians and farmers can develop a calf health plan for free within the University of Nottingham Herd Health Toolkit, where they can receive a bespoke calf health plan based on their current calf management practices.

7.3.5. Chapter 6

Given the findings in previous chapters that environmental temperatures appeared to be associated with both mortality and growth rates, a randomised controlled trial was conducted to explore the effect of both 1kW heat lamps and calf jackets with a factorial study design. The results from the study suggest that whilst calf jackets did not have a significant effect, 1kW heat lamps can have a significant positive impact on growth rates of preweaned calves on GB dairy farms. Environmental temperature was also found to be positively associated with growth rates, which was further reinforced when data from previous research was incorporated within a Bayesian modelling framework. Veterinarians and farmers should

consider the implementation of additional heat sources such as 1kW heat lamps to improve growth rates of calves in colder conditions.

7.3.6. Overall

Whilst there are a large number of potential factors associated with calf health and production outcomes, this research has identified several management factors likely to be associated with several key outcomes, which were subsequently tested in a randomised controlled trial. These factors largely revolve around milk feeding, environment temperatures and the hygiene management practices of both colostrum equipment and calf housing. The impact of environmental temperatures has been further tested, and the provision of supplementary heat source in the form of a 1kW heat lamp has been shown to improve growth rates in a randomised controlled trial.

Whilst conventional statistical techniques are known to perform poorly in high dimensional datasets, there are a number of statistical learning techniques that can provide robust estimates of coefficients and confidence intervals such as the use of regularised regression. In addition to the accurate estimation of effect size for a given variable, the determination of variables likely to impact calf performance on the majority of farms is crucial, and the use of stability selection methods provides a robust estimate of the likely reproducibility of a variable effect within the target population. Used in combination, the use of statistical learning algorithms and stability selection can be utilised as a method of identifying a relatively small set of variables with a large and consistent influence on key animal health and performance outcomes. The *stabiliser* R package is freely available and allows these methods to be easily implemented within the wider research community.

By testing key management factors identified in this thesis as a calf health plan in a randomised controlled setting, it has been shown that the implementation of points from this plan is likely to improve the growth rates and decrease morbidity and mortality rates of calves on dairy farms. A number of farmer and veterinarian focussed tools have been created based on this research, and the tools, health plan and reporting framework are all available through the University of Nottingham Herd Health Toolkit (www.nottingham.ac.uk/herdhealthtoolkit).

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Appendices

Appendix i

Farm questionnaire

Chapter 8 The following section provides the questionnaire utilised in Chapter 3 to determine calf management practices for participating farmers.

Farm information

Farm name _____ Farm ID _____

Farm Address _____

Tel _____ Email _____

CPH number _____ County _____

Farm type: Organic/Conventional Milk record: Yes/No (How often?)

Predominant breed _____

Number of milking cows (average) _____ Number of adult beef/dairy bulls

Calving system: Year round/Spring Block/Autumn block/Multiple block

What date does calving start? _____ When does calving finish? (90%)

Number of cows calved in last 12months _____ Number dairy bull calves born?

Average production (kg/305d) _____

Farm under bTB restrictions: Yes/No

Any other livestock on farm? None/Sheep/Non-dairy beef/Pigs/Poultry/Other

Other livestock within 1 mile? None/Sheep/Dairy cattle/Beef/Pigs/Poultry/Other

Milking system: Robot/Parlour Milking times per day: Two/Three/Other

Main milking housing: Cubicles/Loose yard/Other

Months of year milking cows spent outdoors (>=4hr/d outside) _____

Is your herd closed (no movement of ANY stock onto the farm in last 12months)? Yes/No

If no, what age groups have been bought in? _____

Do you check new arrivals for disease status? _____

Herd disease status/vaccination (IBR/BVD/Johnes/Lepto/Salmonella/mastitis vaccines)?

Disease	Product	Starter course (including age)	Booster details	Test result details

BVD testing: None/Tag and test/Cohort test/BVD free? _____

Are dry cows vaccinated? With what? _____

Manure handling system? _____

If management is different for different groups of calves (i.e. bull vs heifer, beef vs dairy) then please highlight.

Farmer information

Age category_____

Role on farm _____

Number of people involved in day to day care of pre-weaned calves ____

Gender/status of primary youngstock carer(s): Male/Female/Other, Family/Employee

Number of years experience in calf management of primary youngstock carer(s) _____

Contract time: Full time/Part time

How many years ago was previous staff change for youngstock carer? _____

Are any planned in next year? _____

How often does the vet visit the farm? (days) _____

% of routine visits that veterinarian visits/discusses calves _____

Has your vet/other benchmarked your farm's calf health against other farms? _____

How important do you rate the role of the vet in treating calf diseases?

Not important at all Extremely important

1 2 3 4 5 6 7 8 9 10

How important do you rate the role of the vet in preventative calf health management?

Not important at all Extremely important

1 2 3 4 5 6 7 8 9 10

Calving area

How important is calving area management in terms of influencing calf health?

Not important at all Extremely important

1 2 3 4 5 6 7 8 9 10

What is your average dry period length? _____

Is dry cow nutrition formulated by a nutritionist? Y/N

Do you use a calving pen/area? Yes/Sometimes/No

If NO where do they calve? _____

If YES What % of calvings happen in dedicated maternity ONLY pen? _____

How long before calving are cows moved into calving pen/area? _____

Is calving pen/area used exclusively for calving cows? Yes/No (hospital)/No (Quarantine)

Is calving pen/area Individual/group? _____

Close to calving dry cows shared with sick/lame cows? Yes/No

How often is calving area bedded (d)? _____ With _____

How is bedding material stored? _____

Between every birth do you clean calving pens/area? No/Yes (full clean)/Yes (fresh bedding only)

If NO how often cleaned? _____

What disinfection products used? _____

How often is calving pen checked for calving animals? _____

Do you use video/other surveillance to check for calving animals? Yes/No

Difficult calvings? %

No assistance Minor assistance (manual pull) Mechanical assistance (Jack/vet) Caesarean

How long is calf in calving pen for (hours)? Min _____ Max _____ Average

<1hr	1-6hr	6-12hr	12-24hr	24-48hr	>48hr

Calf Health

How do you monitor calf performance (i.e. growth rates, disease monitoring, vet monitoring)? _____

Who is the most influential person to get advice from regarding calf health? _____

Where do you get MOST of your calf health/management information? _____

What is the single biggest challenge you face with your calves? _____

Do you use fevertags? Yes/No/Used to

Calves born per month (including stillbirths) _____

Perceived stillbirths (% dead or died within 24hours) rate _____

Perceived preweaning mortality % (including stillbirths)_____

How do you view this rate? Very High/High/Normal/Low/Very Low

How much would you be willing to invest to reduce mortality by 25%? _____

Perceived average number of cases of pneumonia per month _____

How do you view this rate? Very High/High/Normal/Low/Very Low

Products used to treat _____

Perceived average number of cases of scour per month _____

How do you view this rate? Very High/High/Normal/Low/Very Low

Products used to treat _____

Is there any isolation for sick calves? _____

How much would you be willing to invest to reduce cases of disease (scour/pneumonia) by 25%? _____

If applicable, how long after birth is navel treatment? _____ What product? _____

How is dehorning performed? _____What anaesthetic? _____ Age (weeks) __

NSAID? _____Who is responsible? _____Before/after weaning?

How is castration performed? _____What anaesthetic? _____Age (weeks) __

NSAID? _____Who is responsible? _____Before/after weaning?

How are bull calves dealt with? (Reared on your farm, sold, shot, other)

At what age are dairy bull calves sold off your farm?

Sold for rearing at _____, Sold as stores at _____, Sold as finished animals at ___

Do you have a local approved finishing unit (AFU)? Yes/No/Don't Know

If yes, do you use it? _____Why? _____

Primary outlet for selling bull calves? Direct to market/abattoir, calf marketing scheme, milk buyer scheme, private sale, other Approximate sale price? _____

What marketing opportunities do you have for dairy bull calves? _____

Calf birthweight measured? Yes/No/Sometimes

Do you record all scour/pneumonia/deaths? Yes/No

Have any recent (within last year) calf disease diagnoses been made by a vet? _____

What vaccines/supplements (i.e. Vit E) given to preweaned calves? When?

Product	Age administered (d)	Second administration?

Is there any routine use of antibiotics in calves? _____

Do you have a standard antibiotic treatment plan for:

Disease	Plan	Created by?
Scour		
Pneumonia		

Colostrum

How important do you rate colostrum management in calf health management?

Not important at all Extremely important

1 2 3 4 5 6 7 8 9 10

Is colostrum collected from cows/heifers? _____ How? _____

Strip/Dip/Dry for colostrum collection?

When is colostrum collected from cows? Before/after/during/separate to milking

Clean hands/gloves used for colostrum collection? _____

How is colostrum collection equipment cleaned between cows? _____

How long do you allow calf to suckle on mother? Is this the same as time left in pen?

<1hr	1-6hr	6-12hr	12-24hr	24-48hr	>48hr

% of colostrum type used

Fresh colostrum from dam	
Fresh colostrum from other cow	
Refrigerated colostrum	
Frozen colostrum from other cow	
Pasteurised colostrum	
Acidified colostrum	
Colostrum replacer	

When do you AIM to feed first colostrum to calves (not including suckling if bottle/tube fed)?_____

What is the LONGEST period a calf might go without colostrum? _____

How long until calf gets first colostrum feed (not including suckling if bottle/tube fed)? %

<1hr	1-6hr	6-12hr	12-24hr	24-48hr	>48hr

What quantity? _____

What method is used to feed colostrum? Bucket/bottle/oesophageal feeder/suckle

When would you use oesophageal feeder? Always, Never, if hadn't drunk X amount?

Do you vary colostrum quantity? What is this based on? _____

Is there a second colostrum feed? _____

Quantity? _____Source (bucket/bottle)?_____ When? _____

Is colostrum always first milking? Yes/No

Is colostrum pasteurised? _____ What model/how long for/temp? _____

Stored? Frozen? How long for? _____

Pooled colostrum? Yes/No/Johnes free only

How do you assess colostrum quality? _____

What is your cut off for "Good"/"Bad" Colostrum? _____

If quality not excellent what do you do? Increase volume/use different fresh/use different frozen/other

Do you monitor calf blood samples for immune transfer? _____

How often? (months) _____

Colostrum supplement (<100g IgG) or substitute (>100g IgG) being used? _____

How many days do calves get fed colostrum/transition milk? _____

Different feeding for heifer/bull calves? _____

Milk feeding

How important do you rate milk feeding management in calf health management?

Not important at all Extremely important

1 2 3 4 5 6 7 8 9 10

What milk is fed to calves (%) ?

Whole saleable Antibiotic waste milk Waste milk (non-AB) Milk replacer

Milk feeding protocol

Group	Housing type	Age range (days)	What (milk/powder)	How (i.e. bucket, bottle, auto)	Times/day	Volume/feed	Concentration

How does liquid milk get stored? _____

How long for? _____ Fed warm/cold? Temp? _____

How do milk collection containers get cleaned? _____ How often? ___

How does milk feeding equipment get cleaned? _____ How often? ___

If automatic how often manually calibrate feeders? _____ ad lib/restricted?

How often hoses/teats replaced? _____ Automatic calibration? Yes/No/Unsure

What weight powder per litre? _____ How is powder weight calculated? ____

Any additives to milk replacer (including acidifier)? _____

What powder? _____Cost? _____ Per _____

Age of weaning? _____

What age do calves get free access to water? _____What age dry feed introduced? _

Do you measure dry feed intake? Yes/No

What feeds available? _____ If straw present is it chopped? Yes/No

Do you weigh calves? _____ When? _____

Target preweaning growth rate? _____

Weaning

When is your target for age at first calving? _____

What age do calves of different groups leave farm? _____

What basis weaned? Age/date/feed/intake/weight/other _____

What age/date/feed intake/weight? _____

How is weaning done? Abrupt/Gradual/Step/Other

How are calves moved or regrouped around weaning? _____

Are any other procedures performed at/around weaning? _____

Sexed semen used? _____

If yes, why? _____

If no, why not? Cost/Availability/Conception rates/Other

Building management

How important do you rate building management in calf health management?

Not important at all Extremely important

1 2 3 4 5 6 7 8 9 10

Are newborn pens cleaned between calves? Always/No/Sometimes (how often?)

When are calves moved from newborn pens to group housing by %?

<7d	7-14d	14-21d	21d-28d	28-45d	>45d

Are group pens cleaned between calves? Always/No/Sometimes (how often?)

What bedding is used? _____ How often cleaned out? _____ Bedding added? _____

Disinfected between bedding? _____

Do older cattle share airspace with young calves? Yes/No What maximum age? __

Mean group sizes in each building? _____

Is there movement of people from adult to calf housing? Yes/No

Are there dedicated calf only personnel? _____

Do you use mechanical ventilation (fans)? _____

Are there galebreaker/mesh side covers for any buildings? _____

How often are they used? _____

Are calf jackets used? Who wears them and what %? _____

Colostrum/Milk assessment

Group	Product	Powder weight	Liquid volume (weight)	Made UP to volume?	Fed to how many calves	Temperature	Sealed container?

Building map 1

Group	Ages	Number (Max)	Bedding depth	Bedding wetness	Rain shelter over bed %	Ventilation type (number of fans)	Cobwebs	Ridge type	Shared water	Water over bedding?	Poaching?	Nesting	Ammonia	Water clean

Group	Min/max airspeed human, calf, lying (m/s, internal + external)	Temperature	RH	Lux centre	Temp Back	Temp L	Temp R	Temp F	Temp centre
Outside									

Building map 2

Group	Ages	Number (Max)	Bedding depth	Bedding wetness	Rain shelter over bed %	Ventilation type (number of fans)	Cobwebs	Ridge type	Shared water	Water over bedding?	Poaching?	Nesting	Ammonia	Water clean

Group	Min/max airspeed human, calf, lying (m/s, internal + external)	Temperature	RH	Lux centre	Temp Back	Temp L	Temp R	Temp F	Temp centre

Building map 3

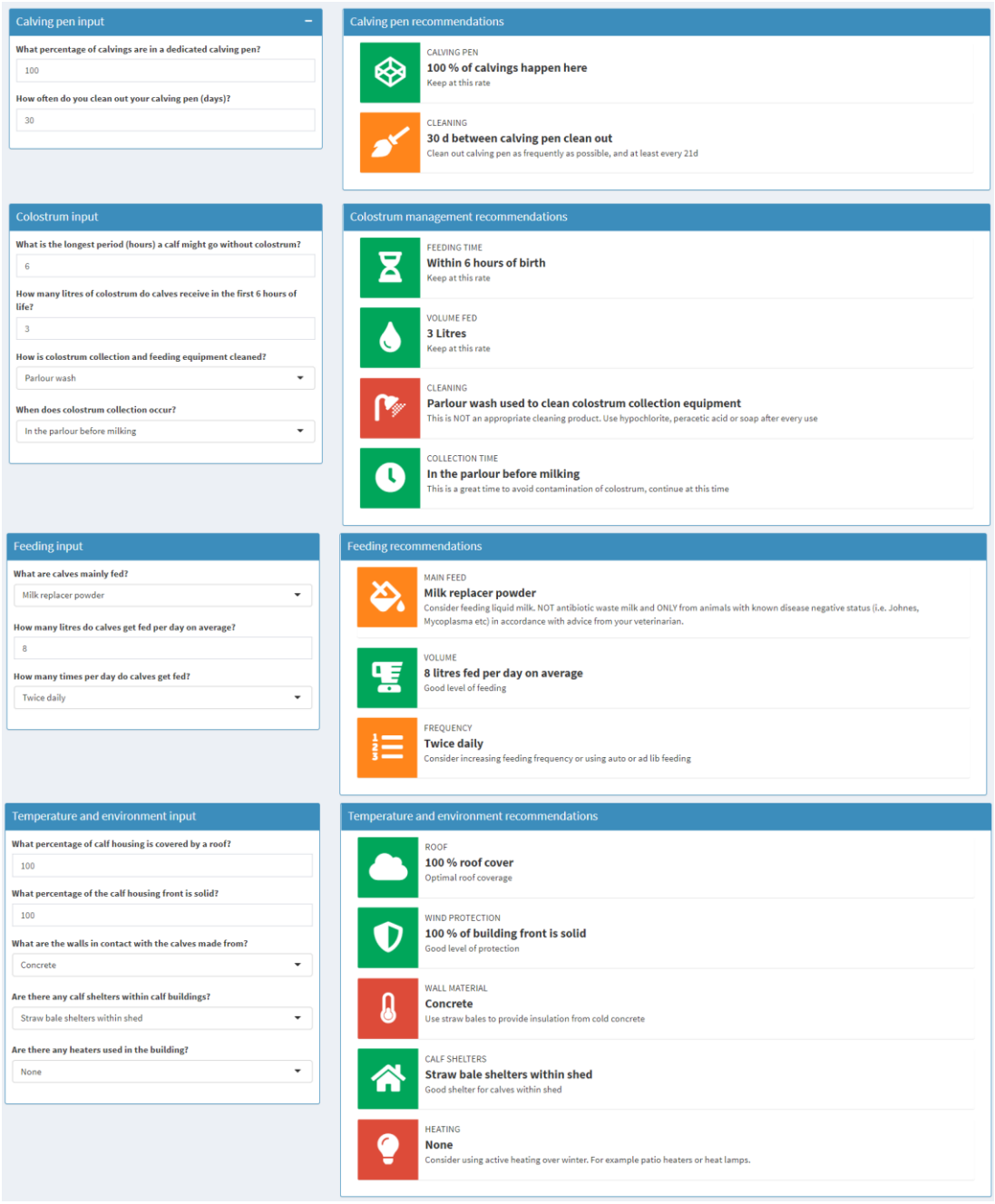
Group	Ages	Number (Max)	Bedding depth	Bedding wetness	Rain shelter over bed %	Ventilation type (number of fans)	Cobwebs	Ridge type	Shared water	Water over bedding?	Poaching?	Nesting	Ammonia	Water clean

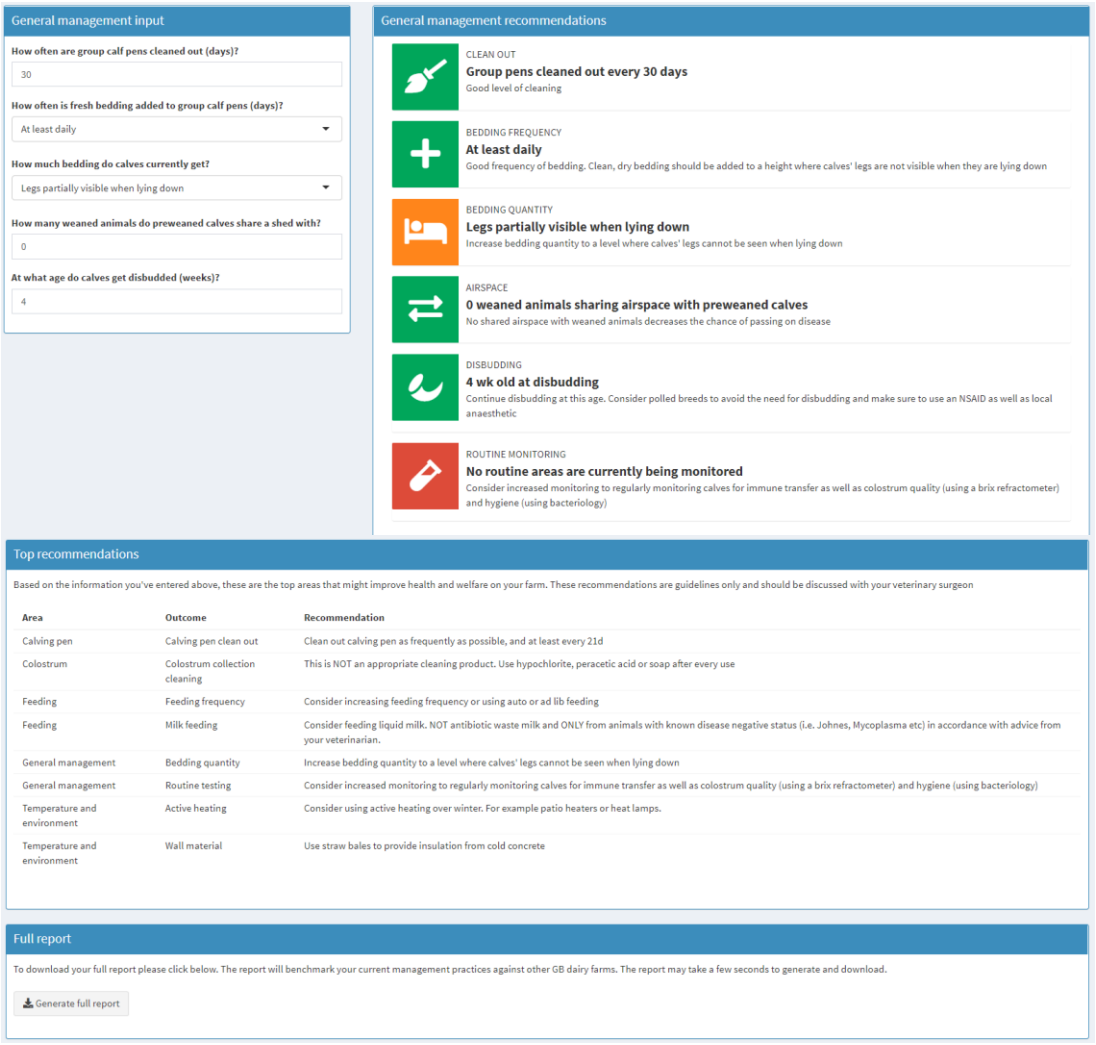
Group	Min/max airspeed human, calf, lying (m/s, internal + external)	Temperature	RH	Lux centre	Temp Back	Temp L	Temp R	Temp F	Temp centre

Appendix ii

Herd health toolkit app demonstration

The following section provides a demonstration of the user experience when using the calf health plan component of the herd health toolkit.




General management input
How often are group calf pens cleaned out (days)?
30
How often is fresh bedding added to group calf pens (days)?
At least daily
How much bedding do calves currently get?
Legs partially visible when lying down
How many weaned animals do preweaned calves share a shed with?
0
At what age do calves get disbudded (weeks)?
4
General management recommendations
CLEAN OUT
Group pens cleaned out every 30 days
Good level of cleaning
BEDDING FREQUENCY
At least daily
Good frequency of bedding. Clean, dry bedding should be added to a height where calves' legs are not visible when they are lying down
BEDDING QUANTITY
Legs partially visible when lying down
Increase bedding quantity to a level where calves' legs cannot be seen when lying down
AIRSPACE
0 weaned animals sharing airspace with preweaned calves
No shared airspace with weaned animals decreases the chance of passing on disease
DISBUDDING
4 wk old at disbudding
Continue disbudding at this age. Consider polled breeds to avoid the need for disbudding and make sure to use an NSAID as well as local anaesthetic
ROUTINE MONITORING
No routine areas are currently being monitored
Consider increased monitoring to regularly monitoring calves for immune transfer as well as colostrum quality (using a brix refractometer) and hygiene (using bacteriology)
Top recommendations
Based on the information you've entered above, these are the top areas that might improve health and welfare on your farm. These recommendations are guidelines only and should be discussed with your veterinary surgeon
Area
Outcome
Recommendation
Calving pen
Calving pen clean out
Clean out calving pen as frequently as possible, and at least every 21d
Colostrum
Colostrum collection cleaning
This is NOT an appropriate cleaning product. Use hypochlorite, peracetic acid or soap after every use
Feeding
Feeding frequency
Consider increasing feeding frequency or using auto or ad lib feeding
Feeding
Milk feeding
Consider feeding liquid milk. NOT antibiotic waste milk and ONLY from animals with known disease negative status (i.e. Johnes, Mycoplasma etc) in accordance with advice from your veterinarian.
General management
Bedding quantity
Increase bedding quantity to a level where calves' legs cannot be seen when lying down
General management
Routine testing
Consider increased monitoring to regularly monitoring calves for immune transfer as well as colostrum quality (using a brix refractometer) and hygiene (using bacteriology)
Temperature and environment
Active heating
Consider using active heating over winter. For example patio heaters or heat lamps.
Temperature and environment
Wall material
Use straw bales to provide insulation from cold concrete
Full report
To download your full report please click below. The report will benchmark your current management practices against other GB dairy farms. The report may take a few seconds to generate and download.
Generate full report

Herd health toolkit app code

The following section provides the code for the herd health toolkit app. This section only provides the outline code, with individual imported modules (such as the calf mortality and colostrum bacteriology etc) being excluded for brevity. The calf health toolkit code is provided in the following section as an example of a single module.

```
source("modules/load_objects.R")
source("modules/global_functions.R")
source("modules/layout.R")
source("modules/getting_started.R")
source("modules/calf/total_protein.R")
source("modules/calf/colostrum_bacteriology.R")
source("modules/calf/calf_mortality_benchmark.R")
source("modules/calf/calf_mortality_forecast.R")
source("modules/calf/calf_dlwg.R")
source("modules/calf/calf_powder.R")
source("modules/cow/amu.R")
source("modules/cow/pat.R")
source("modules/cow/mob_score.R")
source("modules/cow/living_space.R")
source("modules/calf/health_plan.R")
source("modules/downloads.R")

body <- dashboardBody(
  tags$head(includeScript("objects/analytics.js")),
  tabItems(gettingStartedUI("start"),
           tpUI("tp"),
           colostrumBactUI("colostrum_bact"),
           calfMortalityBenchmarkUI("mortality"),
           calfMortalityForecastUI("forecast"),
           calfDLWGUI("dlwg"),
           calfPowderUI("powder"),
           healthPlanUI("health_plan"),
           amuUI("amu"),
           patUI("pat"),
           mobScoreUI("mob_score"),
           livingSpaceUI("living_space"),
           downloadsUI("downloads")
           )
  )

ui <- dashboardPage(
  skin = "green",
  header,
  sidebar,
  body)

server <- function(input, output, session) {

  callModule(gettingStartedServer, "start")
  callModule(tpServer, "tp")
  callModule(colostrumBactServer, "colostrum_bact")
  callModule(calfMortalityBenchmarkServer, "mortality")
  callModule(calfMortalityForecastServer, "forecast")
  callModule(calfDLWGServer, "dlwg")
  callModule(calfPowderServer, "powder")
  callModule(healthPlanServer, "health_plan")
  callModule(amuServer, "amu")
  callModule(patServer, "pat")
  callModule(mobScoreServer, "mob_score")
  callModule(livingSpaceServer, "living_space")
  callModule(downloadsServer, "downloads")
}

shinyApp(ui, server)
```

Calf health plan module code

This section provides the code for the user interface and server of the calf health plan module of the herd health toolkit.

```
healthPlanUI <- function(id) {

  ns <- NS(id)

  tabItem(tabName = "health_plan",
          br(),
          fluidRow(
            box(title = tags$b("Getting started"),
    solidHeader = TRUE, status = "primary", width = 12, collapsible = TRUE,
                "The calf health plan is a free tool to help improve the health and welfare of calves on UK farms.",
                br(),
                br(),
                "First enter your current management details in the following boxes on the left. A traffic light coded recommendation
summary is provided for each section on the right. A full report of your custom calf health plan is available to download at the bottom
of the page."
            )
          ),
          fluidRow(
            box(title = "Calving pen input", solidHeader = TRUE, status = "primary", collapsible = TRUE, width=4,
                numericInput(inputId = ns("percent_use_calving_pen_mean"),
                             "What percentage of calvings are in a dedicated calving pen?",
                             value = 100),
                numericInput(ns("calving_pen_clean_interval_d"),
                             "How often do you clean out your calving pen (days)?",
                             value = 30)
            ),
            box(title = "Calving pen recommendations", solidHeader = TRUE, status = "primary", width=8,
                uiOutput(ns("percent_use_calving_pen_mean")),
                uiOutput(ns("calving_pen_clean_interval_d"))
            )
          ),
          fluidRow(
            box(title = "Colostrum input", solidHeader = TRUE, status = "primary", width=4,
                numericInput(inputId = ns("longest_colostrum_within_hr"),
                             "What is the longest period (hours) a calf might go without colostrum?",
                             value = 6),
                numericInput(inputId = ns("colostrum_quantity"),
                             "How many litres of colostrum do calves receive in the first 6 hours of life?",
                             value = 3),
                selectInput(inputId = ns("colostrum_collection_cleaned"),
                            "How is colostrum collection and feeding equipment cleaned?",
                            choices = c("Parlour wash",
                                        "Peracetic acid",
```

```
                                "Hypochlorite",
                                "Soap",
                                "Not done",
                                selected = NULL)),
        selectInput(inputId = ns("colostrum_colection_time"),
                    "When does colostrum collection occur?",
                    choices = c("In the parlour before milking",
                                "In the parlour during milking",
                                "In the parlour after milking",
                                "Separate to milking",
                                "Not done",
                                selected = FALSE))
    ),
    box(title = "Colostrum management recommendations", solidHeader = TRUE, status = "primary", width=8,
        uiOutput(ns("longest_colostrum_within_hr")),
        uiOutput(ns("colostrum_quantity")),
        uiOutput(ns("colostrum_collection_cleaned")),
        uiOutput(ns("colostrum_colection_time"))
    )
  ),
  fluidRow(
    box(title = "Feeding input", solidHeader = TRUE, status = "primary",  width=4,
        selectInput(inputId = ns("main_feed"),
                    "What are calves mainly fed?",
                    choices = c("Milk replacer powder",
                                "Liquid milk",
                                "Other")),
        numericInput(inputId = ns("mean_fed_per_day"),
                     "How many litres do calves get fed per day on average?",
                     value = 8),
        selectInput(inputId = ns("mean_freq_per_day"),
                    "How many times per day do calves get fed?",
                    choices = c("Twice daily",
                                "Once daily",
                                "Three times daily",
                                "Auto feeder",
                                "Ad lib"))
    ),
    box(title = "Feeding recommendations", solidHeader = TRUE, status = "primary", width=8,
        uiOutput(ns("main_feed")),
        uiOutput(ns("mean_fed_per_day")),
        uiOutput(ns("mean_freq_per_day"))
    )
  ),
  fluidRow(
    box(title = "Temperature and environment input", solidHeader = TRUE, status = "primary",  width=4,
        numericInput(inputId = ns("group1_rain_shelter_pct"),
                     "What percentage of calf housing is covered by a roof?",
```

```
                value = 100),
      numericInput(inputId = ns("mean_solid_front_pct"),
                "What percentage of the calf housing front is solid?",
                value = 100),
      selectInput(inputId = ns("group1_mean_wall_temp"),
                "What are the walls in contact with the calves made from?",
                choices = c("Concrete",
                            "Wood",
                            "Plastic",
                            "Metal hurdle",
                            "Straw",
                            "Other")),
      selectInput(inputId = ns("calf_shelters"),
                "Are there any calf shelters within calf buildings?",
                choices = c("Straw bale shelters within shed",
                            "Hutches within shed",
                            "Hutches outside",
                            "No calf shelters")),
      selectInput(inputId = ns("active_heating"),
                "Are there any heaters used in the building?",
                choices = c("None",
                            "Heat lamps or heaters used"))
  ),
  box(title = "Temperature and environment recommendations", solidHeader = TRUE, status = "primary", width=8,
      uiOutput(ns("group1_rain_shelter_pct")),
      uiOutput(ns("mean_solid_front_pct")),
      uiOutput(ns("group1_mean_wall_temp")),
      uiOutput(ns("calf_shelters")),
      uiOutput(ns("active_heating"))

  )
),
fluidRow(
  box(title = "General management input", solidHeader = TRUE, status = "primary",  width=4,
      numericInput(inputId = ns("group_cleaning_freq_d"),
                "How often are group calf pens cleaned out (days)?",
                value = 30),
      selectInput(inputId = ns("group_bedding_added_d"),
                "How often is fresh bedding added to group calf pens (days)?",
                choices = c("At least daily",
                            "Less than daily",
                            "Not done")),
      selectInput(inputId = ns("nesting"),
                "How much bedding do calves currently get?",
                choices = c("Legs partially visible when lying down",
                            "Legs visible when lying down",
                            "Legs not visible when lying down")),
      numericInput(inputId = ns("mean_weaned_shed"),
```

```
                        "How many weaned animals do preweaned calves share a shed with?",
                        value = 0),
            numericInput(inputId = ns("disbudding_age_wk"),
                        "At what age do calves get disbudded (weeks)?",
                        value = 4)
        ),
        box(title = "General management recommendations", solidHeader = TRUE, status = "primary", width=8,
            uiOutput(ns("group_cleaning_freq_d")),
            uiOutput(ns("group_bedding_added_d")),
            uiOutput(ns("nesting")),
            uiOutput(ns("mean_weaned_shed")),
            uiOutput(ns("disbudding_age_wk")),
            uiOutput(ns("routine_testing"))
        )
      ),
      box(title = "Testing", solidHeader = TRUE, status = "primary",
          "What routine testing is currently underway? Tick all that apply.",
          checkboxGroupInput(inputId = ns("routine_testing"),
                             label = "",
                             choices = c("Calf blood samples for total proteins",
                                         "Colostrum quality with refractometer",
                                         "Colostrum bacteriology"),
                             inline = TRUE)
      ),
      box(title = "Areas to improve", solidHeader = TRUE, status = "primary",
          "Which areas are you interested in improving? Tick all that apply.",
          checkboxGroupInput(inputId = ns("interested_in"),
                             label = "",
                             choices = c("Scour" = "scour",
                                         "Pneumonia" = "pneum",
                                         "Mortality" = "mort",
                                         "Growth rates" = "dlwg"),
                             selected = c("scour", "pneum", "mort", "dlwg"),
                             inline = TRUE)
      ),
      fluidRow(box(title = "Top recommendations", solidHeader = TRUE, status = "primary", width = 12,
                   "Based on the information you've entered above, these are the top areas that might improve health and welfare on
your farm. These recommendations are guidelines only and should be discussed with your veterinary surgeon",
                   br(),
                   br(),
                   tableOutput(outputId = ns("top_rec")),
                   br()
      )
      ),
      fluidRow(box(title = "Full report", solidHeader = TRUE, status = "primary", width = 12,
                   "To download your full report please click below. The report will benchmark your current management practices
against other GB dairy farms. The report may take a few seconds to generate and download.",
                   br(),
```

```
                    br(),
                    downloadButton(ns("health_plan_report"), "Generate full report"),
                    br(),
                    br()
          )
          )
  )
}

healthPlanServer <- function(input, output, session){

  ### Health plan
  table <- reactive({
    tibble(percent_use_calving_pen_mean = as.numeric(input$percent_use_calving_pen_mean),
           calving_pen_clean_interval_d = as.numeric(input$calving_pen_clean_interval_d),
           longest_colostrum_within_hr = as.numeric(input$longest_colostrum_within_hr),
           colostrum_quantity = as.numeric(input$colostrum_quantity),
           colostrum_collection_cleaned = as.character(input$colostrum_collection_cleaned),
           colostrum_colection_time = as.character(input$colostrum_colection_time),
           main_feed = as.character(input$main_feed),
           mean_fed_per_day = as.numeric(input$mean_fed_per_day),
           mean_freq_per_day = as.character(input$mean_freq_per_day),
           group1_rain_shelter_pct = as.numeric(input$group1_rain_shelter_pct),
           mean_solid_front_pct = as.numeric(input$mean_solid_front_pct),
           group1_mean_wall_temp = as.character(input$group1_mean_wall_temp),
           group_cleaning_freq_d = as.numeric(input$group_cleaning_freq_d),
           group_bedding_added_d = as.character(input$group_bedding_added_d),
           calf_shelters = as.character(input$calf_shelters),
           active_heating = as.character(input$active_heating),
           nesting = as.character(input$nesting),
           mean_weaned_shed = as.numeric(input$mean_weaned_shed),
           disbudding_age_wk = as.numeric(input$disbudding_age_wk),
           routine_testing = as.character(paste(input$routine_testing, collapse = ", ")),
           interested_in = as.character(paste(input$interested_in, collapse = ", "))
    ) %>%
      mutate(colour_percent_use_calving_pen_mean = case_when(percent_use_calving_pen_mean >= 100 ~ "green",
                                                             percent_use_calving_pen_mean >=80 & percent_use_calving_pen_mean < 100 ~
"orange",
                                                             percent_use_calving_pen_mean < 80 ~ "red"),
             advice_percent_use_calving_pen_mean = case_when(percent_use_calving_pen_mean >= 100 ~ "Keep at this rate",
                                                             percent_use_calving_pen_mean < 100 ~ "Aim to calve all calves in a
dedicated calving pen"),
             colour_calving_pen_clean_interval_d = case_when(calving_pen_clean_interval_d <= 21 ~ "green",
                                                             calving_pen_clean_interval_d >21 & calving_pen_clean_interval_d <= 30 ~
"orange",
                                                             calving_pen_clean_interval_d > 30 ~ "red"),
             advice_calving_pen_clean_interval_d = case_when(calving_pen_clean_interval_d <= 21 ~ "Keep at this rate",
```

```
                                                    calving_pen_clean_interval_d > 21 ~ "Clean out calving pen as frequently as
possible, and at least every 21d"),
            colour_longest_colostrum_within_hr = case_when(longest_colostrum_within_hr <= 6 ~ "green",
                                                           longest_colostrum_within_hr >6 & longest_colostrum_within_hr <= 8 ~
"orange",
                                                           longest_colostrum_within_hr > 8 ~ "red"),
            advice_longest_colostrum_within_hr = case_when(longest_colostrum_within_hr <= 6 ~ "Keep at this rate",
                                                           longest_colostrum_within_hr > 6 ~ "Aim for calves to receive colostrum as
soon as possible, and always within 6 hours of birth"),
            colour_colostrum_quantity = case_when(colostrum_quantity >= 3 & colostrum_quantity <= 4 ~ "green",
                                                  colostrum_quantity > 4  & colostrum_quantity < 5 ~ "orange",
                                                  colostrum_quantity <3 & colostrum_quantity >= 2.5 ~ "orange",
                                                  colostrum_quantity <2.5 | colostrum_quantity >= 5 ~ "red"),
            advice_colostrum_quantity = case_when(colostrum_quantity >= 3 & colostrum_quantity <= 4 ~ "Keep at this rate",
                                                  colostrum_quantity > 4  & colostrum_quantity < 5 ~ "Consider reducing to 3-4L in
first feed or providing over 2 feeds (6 hours apart)",
                                                  colostrum_quantity >=5 ~ "Reduce colostrum volume to 3-4L in first feed",
                                                  colostrum_quantity <3 & colostrum_quantity >= 2.5 ~ "Increase colostrum volume to 3-
4L in first feed",
                                                  colostrum_quantity <2.5 ~ "Increase colostrum feed to 3-4L in first feed"),
            colour_colostrum_collection_cleaned = case_when(colostrum_collection_cleaned == "Hypochlorite" |
                                                              colostrum_collection_cleaned == "Peracetic acid" |
                                                              colostrum_collection_cleaned == "Soap" ~ "green",
                                                            colostrum_collection_cleaned == "Parlour wash" |
                                                              colostrum_collection_cleaned == "Water" ~ "red",
                                                            colostrum_collection_cleaned == "Not done" ~ "red"),
            advice_colostrum_collection_cleaned = case_when(colostrum_collection_cleaned == "Hypochlorite" |
                                                              colostrum_collection_cleaned == "Peracetic acid" |
                                                              colostrum_collection_cleaned == "Soap" ~ "This is an appropriate cleaning
product, make sure to clean colostrum collection and feeding equipment after each use",
                                                            colostrum_collection_cleaned == "Parlour wash" |
                                                              colostrum_collection_cleaned == "Water" ~ "This is NOT an appropriate
cleaning product. Use hypochlorite, peracetic acid or soap after every use",
                                                            colostrum_collection_cleaned == "Not done" ~ "Use hypochlorite, peracetic
acid or soap to clean out colostrum collection equipment after every use"),
            colour_colostrum_colection_time = case_when(colostrum_colection_time == "In the parlour before milking" ~ "green",
                                                        colostrum_colection_time == "In the parlour during milking" ~ "red",
                                                        colostrum_colection_time == "In the parlour after milking" ~ "red",
                                                        colostrum_colection_time == "Separate to milking" ~ "red",
                                                        colostrum_colection_time == "Not done" ~ "red"),
            advice_colostrum_colection_time = case_when(colostrum_colection_time == "In the parlour before milking" ~ "This is a great
time to avoid contamination of colostrum, continue at this time",
                                                        colostrum_colection_time == "In the parlour during milking" ~ "High risk of
contamination when collecting during milking, change to collect colostrum in parlour before milking",
                                                        colostrum_colection_time == "In the parlour after milking" ~ "High risk of
contamination when collecting after milking, change to collect colostrum in parlour before milking",
                                                        colostrum_colection_time == "Separate to milking" ~ "High risk of contamination
when collecting separate to milking, change to collect colostrum in parlour before milking",
```

```
                                                    colostrum_colection_time == "Not done" ~ "Consider collecting colostrum to feed
to calves. Collect in the parlour before milking"),
             colour_main_feed = case_when(main_feed == "Milk replacer powder" ~ "orange",
                                          main_feed == "Liquid milk" ~ "green",
                                          main_feed == "Other" ~ "red"),
             advice_main_feed = case_when(main_feed == "Milk replacer powder" ~ "Consider feeding liquid milk. NOT antibiotic waste milk
and ONLY from animals with known disease negative status (i.e. Johnes, Mycoplasma etc) in accordance with advice from your
veterinarian.",
                                          main_feed == "Liquid milk" ~ "Continue feeding liquid milk. NOT antibiotic waste milk and ONLY
from animals with known disease negative status (i.e. Johnes, Mycoplasma etc) in accordance with advice from your veterinarian.",
                                          main_feed == "Other" ~ "Feed milk replacer powder or preferably liquid milk. NOT antibiotic
waste milk and ONLY from animals with known disease negative status (i.e. Johnes, Mycoplasma etc) in accordance with advice from your
veterinarian."),
             colour_mean_fed_per_day = case_when(mean_fed_per_day <= 5 ~ "red",
                                                 mean_fed_per_day >5 & mean_fed_per_day <= 6 ~ "orange",
                                                 mean_fed_per_day > 6 ~ "green"),
             advice_mean_fed_per_day = case_when(mean_fed_per_day <= 5 ~ "Low level being fed. Increase quantity fed to at least 6-8
litres per day",
                                                 mean_fed_per_day >5 & mean_fed_per_day <= 6 ~ "Increase quantity fed to at least 6-8
litres per day",
                                                 mean_fed_per_day > 6 ~ "Good level of feeding"),
             colour_mean_freq_per_day = case_when(mean_freq_per_day == "Once daily" ~ "red",
                                                  mean_freq_per_day == "Twice daily" ~ "orange",
                                                  mean_freq_per_day == "Three times daily" ~ "green",
                                                  mean_freq_per_day == "Auto feeder" ~ "green",
                                                  mean_freq_per_day == "Ad lib" ~ "green"),
             advice_mean_freq_per_day = case_when(mean_freq_per_day == "Once daily" ~ "Increase feeding frequency to at least twice per
day",
                                                  mean_freq_per_day == "Twice daily" ~ "Consider increasing feeding frequency or using
auto or ad lib feeding",
                                                  mean_freq_per_day == "Three times daily" ~ "Good feeding frequency",
                                                  mean_freq_per_day == "Auto feeder" ~ "Auto feeding allows good feed frequency",
                                                  mean_freq_per_day == "Ad lib" ~ "Ad lib feeding allows good feed frequency"),
             colour_group1_rain_shelter_pct = case_when(group1_rain_shelter_pct < 95 ~ "red",
                                                        group1_rain_shelter_pct >= 95 & group1_rain_shelter_pct <100  ~ "orange",
                                                        group1_rain_shelter_pct == 100 ~ "green"),
             advice_group1_rain_shelter_pct = case_when(group1_rain_shelter_pct < 95 ~ "Increase coverage of calf accomodation to ensure
100% roof cover",
                                                        group1_rain_shelter_pct >= 95 & group1_rain_shelter_pct <100  ~ "Increase
coverage of calf accomodation to ensure 100% roof cover",
                                                        group1_rain_shelter_pct == 100 ~ "Optimal roof coverage"),
             colour_mean_solid_front_pct = case_when(mean_solid_front_pct < 50 ~ "red",
                                                     mean_solid_front_pct >= 50 & mean_solid_front_pct <75  ~ "orange",
                                                     mean_solid_front_pct >= 75 ~ "green"),
             advice_mean_solid_front_pct = case_when(mean_solid_front_pct < 50 ~ "Ensure front and sides of building is protected from
wind",
                                                     mean_solid_front_pct >= 50 & mean_solid_front_pct <75  ~ "Consider increasing level
of solid front to 75% to further protect calves from wind",
```

```
                                                    mean_solid_front_pct >= 75 ~ "Good level of protection"),
            colour_group1_mean_wall_temp = case_when(group1_mean_wall_temp == "Concrete" ~ "red",
                                                     group1_mean_wall_temp == "Wood"  ~ "orange",
                                                     group1_mean_wall_temp == "Plastic"  ~ "orange",
                                                     group1_mean_wall_temp == "Metal hurdle"  ~ "orange",
                                                     group1_mean_wall_temp == "Straw"  ~ "green",
                                                     group1_mean_wall_temp == "Other"  ~ "orange"),
            advice_group1_mean_wall_temp = case_when(group1_mean_wall_temp == "Concrete" ~ "Use straw bales to provide insulation from
cold concrete",
                                                     group1_mean_wall_temp == "Wood"  ~ "Consider straw bales to provide insulation
from wood, ensure thorough cleaning",
                                                     group1_mean_wall_temp == "Plastic"  ~ "Regularly bed with clean straw to ensure
good insulation",
                                                     group1_mean_wall_temp == "Metal hurdle"  ~ "Regularly bed with clean straw to
ensure good insulation",
                                                     group1_mean_wall_temp == "Straw"  ~ "Good insulation",
                                                     group1_mean_wall_temp == "Other"  ~ "Regularly bed with clean straw to ensure good
insulation"),
            colour_group_cleaning_freq_d = case_when(group_cleaning_freq_d > 30  ~ "red",
                                                     group_cleaning_freq_d <= 30  ~ "green"),
            advice_group_cleaning_freq_d = case_when(group_cleaning_freq_d > 30  ~ "Clean out as frequently as possible, at least once
every 30 days",
                                                     group_cleaning_freq_d <= 30  ~ "Good level of cleaning"),
            colour_group_bedding_added_d = case_when(group_bedding_added_d == "At least daily" ~ "green",
                                                     group_bedding_added_d == "Less than daily" ~ "red",
                                                     group_bedding_added_d == "Not done" ~ "red"),
            advice_group_bedding_added_d = case_when(group_bedding_added_d == "At least daily" ~ "Good frequency of bedding. Clean, dry
bedding should be added to a height where calves' legs are not visible when they are lying down",
                                                     group_bedding_added_d == "Less than daily" ~ "Add fresh dry bedding at least
daily. Clean, dry bedding should be added to a height where calves' legs are not visible when they are lying down",
                                                     group_bedding_added_d == "Not done" ~ "Add fresh dry bedding at least daily.
Clean, dry bedding should be added to a height where calves' legs are not visible when they are lying down"),
            colour_active_heating = case_when(active_heating == "None" ~ "red",
                                              active_heating == "Heat lamps or heaters used" ~ "green"),
            advice_active_heating = case_when(active_heating == "None" ~ "Consider using active heating over winter. For example patio
heaters or heat lamps.",
                                              active_heating == "Heat lamps or heaters used" ~ "Continue using heaters over winter"),
            colour_calf_shelters = case_when(calf_shelters == "Straw bale shelters within shed" ~ "green",
                                             calf_shelters == "Hutches within shed" ~ "orange",
                                             calf_shelters == "Hutches outside" ~ "red",
                                             calf_shelters == "No calf shelters" ~ "red"),
            advice_calf_shelters = case_when(calf_shelters == "Straw bale shelters within shed" ~ "Good shelter for calves within
shed",
                                             calf_shelters == "Hutches within shed" ~ "Some shelter for calves within shed. Ensure
shelters are well insulated",
                                             calf_shelters == "Hutches outside" ~ "Ensure hutches have roof coverage or move calves
into building with straw bale shelters",
```

```
                                                calf_shelters == "No calf shelters" ~ "Ensure calves have small shelters within housing.
Straw bale shelters can be a cheap method of acheiving this"),
            colour_mean_weaned_shed = case_when(mean_weaned_shed == 0 ~ "green",
                                                mean_weaned_shed >0 & mean_weaned_shed <= 5  ~ "orange",
                                                mean_weaned_shed > 5  ~ "red"),
            advice_mean_weaned_shed = case_when(mean_weaned_shed == 0 ~ "No shared airspace with weaned animals decreases the chance of
passing on disease",
                                                mean_weaned_shed >0 & mean_weaned_shed <= 5  ~ "House preweaned animals in a separate
airspace to weaned animals to decrease the chance of passing on disease",
                                                mean_weaned_shed > 5  ~ "House preweaned animals in a separate airspace to weaned
animals to decrease the chance of passing on disease"),
            colour_nesting = case_when(nesting == "Legs visible when lying down" ~ "red",
                                       nesting == "Legs partially visible when lying down" ~ "orange",
                                       nesting == "Legs not visible when lying down" ~ "green"),
            advice_nesting = case_when(nesting == "Legs visible when lying down" ~ "Increase bedding quantity to a level where calves'
legs cannot be seen when lying down",
                                       nesting == "Legs partially visible when lying down" ~ "Increase bedding quantity to a level
where calves' legs cannot be seen when lying down",
                                       nesting == "Legs not visible when lying down" ~ "Continue bedding at this level"),
            colour_disbudding_age_wk = case_when(disbudding_age_wk <= 4 ~ "green",
                                                 disbudding_age_wk >4 & disbudding_age_wk <= 6  ~ "orange",
                                                 disbudding_age_wk > 6  ~ "red"),
            advice_disbudding_age_wk = case_when(disbudding_age_wk <= 4 ~ "Continue disbudding at this age. Consider polled breeds to
avoid the need for disbudding and make sure to use an NSAID as well as local anaesthetic",
                                                 disbudding_age_wk >4 & disbudding_age_wk <= 6  ~ "Disbud calves at an earlier age,
preferably at 4 weeks or younger. Consider polled breeds to avoid the need for disbudding and make sure to use an NSAID as well as local
anaesthetic",
                                                 disbudding_age_wk > 6  ~ "Disbud calves at an earlier age, preferably at 4 weeks or
younger. Consider polled breeds to avoid the need for disbudding and make sure to use an NSAID as well as local anaesthetic")
            ,
            colour_routine_testing = case_when(grepl("Calf blood samples for total proteins", routine_testing) &
                                                 grepl("Colostrum quality with refractometer", routine_testing) &
                                                 grepl("Colostrum bacteriology", routine_testing) ~ "green",
                                               grepl("Calf blood samples for total proteins", routine_testing) |
                                                 grepl("Colostrum quality with refractometer", routine_testing) |
                                                 grepl("Colostrum bacteriology", routine_testing) ~ "orange",
                                               TRUE ~ "red"),
            advice_routine_testing =case_when(grepl("Calf blood samples for total proteins", routine_testing) &
                                                grepl("Colostrum quality with refractometer", routine_testing) &
                                                grepl("Colostrum bacteriology", routine_testing) ~ "Continue routine monitoring",
                                              grepl("Calf blood samples for total proteins", routine_testing) |
                                                grepl("Colostrum quality with refractometer", routine_testing) |
                                                grepl("Colostrum bacteriology", routine_testing) ~ "Consider increased monitoring to
regularly monitoring calves for immune transfer as well as colostrum quality (using a brix refractometer) and hygiene (using
bacteriology)",
                                              TRUE ~ "Consider increased monitoring to regularly monitoring calves for immune transfer
as well as colostrum quality (using a brix refractometer) and hygiene (using bacteriology)"),
            routine_testing = case_when(routine_testing == "" ~ "No routine areas",
```

```
                                        TRUE ~ routine_testing),
            routine_testing = sub(",([^,]*)$"," and\\1", routine_testing),
            interested_in = sub(",([^,]*)$"," and\\1", interested_in)
    )
})

top_rec <- reactive({
  colour_list <- table() %>%
    select(contains("colour")) %>%
    gather(variable, colour) %>%
    mutate(variable = str_remove(variable, "colour_"))

  advice_list <- table() %>%
    select(contains("advice")) %>%
    gather(variable, advice) %>%
    mutate(variable = str_remove(variable, "advice_"))

  df_list <- table() %>%
    select(-contains("advice"), -contains("colour")) %>%
    gather(variable, value) %>%
    left_join(variable_list) %>%
    left_join(colour_list) %>%
    left_join(advice_list)

  df_list %>%
    filter(colour != "green") %>%
    filter(outcome %in% input$interested_in |
             outcome == "all") %>%
    arrange(ranking, desc(colour)) %>%
    select(variable, full_name, area) %>%
    distinct() %>%
    head(n=10) %>%
    left_join(advice_list) %>%
    select(area, full_name, advice) %>%
    rename("Outcome" = "full_name",
           "Recommendation" = "advice",
           "Area" = "area") %>%
    arrange(Area)
})

output$top_rec <- renderTable({
  top_rec()
})

output$percent_use_calving_pen_mean <- renderUI({
  value = table() %>% select(percent_use_calving_pen_mean) %>%
    mutate(percent_use_calving_pen_mean = paste(percent_use_calving_pen_mean, "% of calvings happen here", ""))
  subtitle = table() %>% select(advice_percent_use_calving_pen_mean)
```

```
    colour = table() %>% select(colour_percent_use_calving_pen_mean)

    infoBox(value = value, title = "Calving pen", subtitle = subtitle,
            icon = icon(name = "codepen"), color=colour,width = 12)
  })

  output$calving_pen_clean_interval_d <- renderUI({
    value = table() %>% select(calving_pen_clean_interval_d) %>%
      mutate(calving_pen_clean_interval_d = paste(calving_pen_clean_interval_d, "d between calving pen clean out", "")) %>%
      pull()

    subtitle = table() %>% select(advice_calving_pen_clean_interval_d)
    colour = table() %>% select(colour_calving_pen_clean_interval_d)

    infoBox(value = value, title = "Cleaning", subtitle = subtitle, icon = icon("broom"), color=colour, width=12)
  })


  output$longest_colostrum_within_hr <- renderUI({
    value = table() %>% select(longest_colostrum_within_hr) %>%
      mutate(longest_colostrum_within_hr = paste("Within", longest_colostrum_within_hr, "hours of birth", " ")) %>%
      pull()

    subtitle = table() %>% select(advice_longest_colostrum_within_hr)
    colour = table() %>% select(colour_longest_colostrum_within_hr)

    infoBox(value = value, title = "Feeding time", subtitle = subtitle, icon = icon("hourglass-half"), color=colour, width=12)
  })

  output$colostrum_quantity <- renderUI({
    value = table() %>% select(colostrum_quantity) %>%
      mutate(colostrum_quantity = paste(colostrum_quantity, "Litres", "")) %>%
      pull()

    subtitle = table() %>% select(advice_colostrum_quantity)
    colour = table() %>% select(colour_colostrum_quantity)

    infoBox(value = value, title = "Volume fed", subtitle = subtitle, icon = icon("tint"), color=colour, width=12)
  })

  output$colostrum_collection_cleaned <- renderUI({
    value = table() %>% select(colostrum_collection_cleaned) %>%
      mutate(colostrum_collection_cleaned = paste(colostrum_collection_cleaned, "used to clean colostrum collection equipment", " "))
%>%
      pull()

    subtitle = table() %>% select(advice_colostrum_collection_cleaned)
    colour = table() %>% select(colour_colostrum_collection_cleaned)
```

```
  infoBox(value = value, title = "Cleaning", subtitle = subtitle, icon = icon("shower"), color=colour, width=12)
})

output$colostrum_colection_time <- renderUI({

  value = table() %>% select(colostrum_colection_time) %>%
    pull()
  subtitle = table() %>% select(advice_colostrum_colection_time) %>%
    pull()
  colour = table() %>% select(colour_colostrum_colection_time) %>%
    pull()

  infoBox(value = value, title = "Collection time", subtitle = subtitle, icon = icon("clock"), color=colour, width=12)
})

output$main_feed <- renderUI({

  value = table() %>% select(main_feed) %>%
    pull()
  subtitle = table() %>% select(advice_main_feed) %>%
    pull()
  colour = table() %>% select(colour_main_feed) %>%
    pull()

  infoBox(value = value, title = "Main feed", subtitle = subtitle, icon = icon("fill-drip"), color=colour, width=12)
})

output$mean_fed_per_day <- renderUI({


  value = table() %>% select(mean_fed_per_day) %>%
    mutate(value = paste(mean_fed_per_day, "litres fed per day on average", "")) %>%
    pull()

  subtitle = table() %>% select(advice_mean_fed_per_day) %>%
    pull()
  colour = table() %>% select(colour_mean_fed_per_day) %>%
    pull()

  infoBox(value = value, title = "Volume", subtitle = subtitle, icon = icon("blender"), color=colour, width=12)
})

output$mean_freq_per_day <- renderUI({

  value = table() %>% select(mean_freq_per_day) %>%
    pull()
```

```
  subtitle = table() %>% select(advice_mean_freq_per_day) %>%
    pull()
  colour = table() %>% select(colour_mean_freq_per_day) %>%
    pull()

  infoBox(value = value, title = "Frequency", subtitle = subtitle, icon = icon("list-ol"), color=colour, width=12)
})

output$group1_rain_shelter_pct <- renderUI({
  value = table() %>% select(group1_rain_shelter_pct) %>%
    mutate(group1_rain_shelter_pct = paste(group1_rain_shelter_pct, "% roof cover", "")) %>%
    pull()

  subtitle = table() %>% select(advice_group1_rain_shelter_pct) %>%
    pull()
  colour = table() %>% select(colour_group1_rain_shelter_pct) %>%
    pull()

  infoBox(value = value, title = "Roof", subtitle = subtitle, icon = icon("cloud"), color=colour, width=12)
})

output$mean_solid_front_pct <- renderUI({

  value = table() %>% select(mean_solid_front_pct) %>%
    mutate(mean_solid_front_pct = paste(mean_solid_front_pct, "% of building front is solid", "")) %>%
    pull()

  subtitle = table() %>% select(advice_mean_solid_front_pct) %>%
    pull()
  colour = table() %>% select(colour_mean_solid_front_pct) %>%
    pull()

  infoBox(value = value, title = "Wind protection", subtitle = subtitle, icon = icon("shield-alt"), color=colour, width=12)
})

output$group1_mean_wall_temp <- renderUI({

  value = table() %>% select(group1_mean_wall_temp) %>%
    pull()
  subtitle = table() %>% select(advice_group1_mean_wall_temp) %>%
    pull()
  colour = table() %>% select(colour_group1_mean_wall_temp) %>%
    pull()

  infoBox(value = value, title = "Wall material", subtitle = subtitle, icon = icon("thermometer-quarter"), color=colour, width=12)
})
```

```
output$group_cleaning_freq_d <- renderUI({

  value = table() %>% select(group_cleaning_freq_d) %>%
    mutate(group_cleaning_freq_d = paste("Group pens cleaned out every ", group_cleaning_freq_d, "days", "")) %>%
    pull()
  subtitle = table() %>% select(advice_group_cleaning_freq_d) %>%
    pull()
  colour = table() %>% select(colour_group_cleaning_freq_d) %>%
    pull()

  infoBox(value = value, title = "Clean out", subtitle = subtitle, icon = icon("broom"), color = colour, width = 12)
})

output$group_bedding_added_d <- renderUI({
  value = table() %>% select(group_bedding_added_d) %>%
    pull()
  subtitle = table() %>% select(advice_group_bedding_added_d) %>%
    pull()
  colour = table() %>% select(colour_group_bedding_added_d) %>%
    pull()

  infoBox(value = value, title = "Bedding frequency", subtitle = subtitle, icon = icon("plus"), color = colour, width = 12)
})

output$active_heating <- renderUI({
  value = table() %>% select(active_heating) %>%
    pull()
  subtitle = table() %>% select(advice_active_heating) %>%
    pull()
  colour = table() %>% select(colour_active_heating) %>%
    pull()

  infoBox(value = value, title = "Heating", subtitle = subtitle, icon = icon("lightbulb"), color=colour, width=12)
})

output$calf_shelters <- renderUI({
  value = table() %>% select(calf_shelters) %>%
    pull()
  subtitle = table() %>% select(advice_calf_shelters) %>%
    pull()
  colour = table() %>% select(colour_calf_shelters) %>%
    pull()

  infoBox(value = value, title = "Calf shelters", subtitle = subtitle, icon = icon("home"), color = colour, width = 12)
})

output$nesting <- renderUI({
  value = table() %>% select(nesting) %>%
```

```
    pull()
  subtitle = table() %>% select(advice_nesting) %>%
    pull()
  colour = table() %>% select(colour_nesting) %>%
    pull()

  infoBox(value = value, title = "Bedding quantity", subtitle = subtitle, icon = icon("bed"), color = colour, width = 12)
})

output$mean_weaned_shed <- renderUI({
  value = table() %>% select(mean_weaned_shed) %>%
    mutate(mean_weaned_shed = paste(mean_weaned_shed, "weaned animals sharing airspace with preweaned calves", " ")) %>%
    pull()
  subtitle = table() %>% select(advice_mean_weaned_shed) %>%
    pull()
  colour = table() %>% select(colour_mean_weaned_shed) %>%
    pull()

  infoBox(value = value, title = "Airspace", subtitle = subtitle, icon = icon("exchange-alt"), color = colour, width = 12)
})

output$disbudding_age_wk <- renderUI({

  value = table() %>% select(disbudding_age_wk) %>%
    mutate(disbudding_age_wk = paste(disbudding_age_wk, "wk old at disbudding", "")) %>%
    pull()
  subtitle = table() %>% select(advice_disbudding_age_wk) %>%
    pull()
  colour = table() %>% select(colour_disbudding_age_wk) %>%
    pull()

  infoBox(value = value, title = "Disbudding", subtitle = subtitle, icon = icon("affiliatetheme"), color=colour, width=12)
})

output$routine_testing <- renderUI({

  value = table() %>% select(routine_testing) %>%
    mutate(routine_testing = paste(routine_testing, "are currently being monitored", " ")) %>%
    pull()
  subtitle = table() %>% select(advice_routine_testing) %>%
    pull()
  colour = table() %>% select(colour_routine_testing) %>%
    pull()

  infoBox(value = value, title = "Routine monitoring", subtitle = subtitle, icon = icon("vial"), color = colour, width = 12)
})

output$health_plan_report <- downloadHandler(
```

```
    filename = paste0("health_plan_report_", Sys.Date(), ".html"),
    content = function(file) {
      tempReport <- file.path(tempdir(), "health_plan_report.Rmd")
      file.copy("modules/calf/health_plan_report.Rmd", tempReport, overwrite = TRUE)

      tempBact <- file.path(tempdir(), "colostrum_bacteriology.csv")
      file.copy("data/colostrum_bacteriology.csv", tempBact, overwrite = TRUE)

      tempExport <- file.path(tempdir(), "export_data.csv")
      file.copy("data/export_data.csv", tempExport, overwrite = TRUE)

      params <- list(table = table(),
                     top_rec = top_rec())

      rmarkdown::render(tempReport,
                        output_file = file,
                        params = params,
                        envir = new.env(parent = globalenv())
      )
    }
  )
}
```

Automated calf health report demonstration

This section provides an example of the automated calf health report available to download from the herd health toolkit once completing the questionnaire within the calf health plan module.

Calf health report

This calf health report is aimed to provide guidance in reducing the risk of morbidity and mortality and improving health and welfare. This report is intended as guidance only and it is essential that any potential interventions are discussed with your farms' veterinarian before implementation. Benchmarking data is based on a study of 60 dairy farms across Great Britain in 2019.

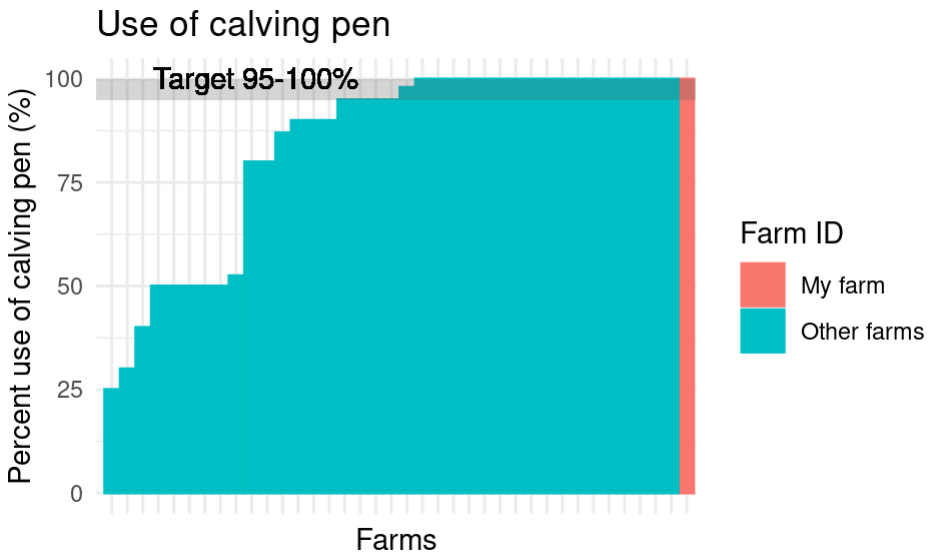
You have said you are most interested in improving scour, pneumonia, mortality and growth rates. To improve these areas, the most important areas to focus on improving are as follows.

Top recommendations:

Area	Outcome	Recommendation
Calving pen	Calving pen clean out	Clean out calving pen as frequently as possible, and at least every 21d
Colostrum	Colostrum collection cleaning	This is NOT an appropriate cleaning product. Use hypochlorite, peracetic acid or soap after every use
Feeding	Feeding frequency	Consider increasing feeding frequency or using auto or ad lib feeding
Feeding	Milk feeding	Consider feeding liquid milk. NOT antibiotic waste milk and ONLY from animals with known disease negative status (i.e. Johnes, Mycoplasma etc) in accordance with advice from your veterinarian.
General management	Bedding quantity	Increase bedding quantity to a level where calves' legs cannot be seen when lying down
General management	Routine testing	Consider increased monitoring to regularly monitoring calves for immune transfer as well as colostrum quality (using a brix refractometer) and hygiene (using bacteriology)
Temperature and environment	Active heating	Consider using active heating over winter. For example patio heaters or heat lamps.
Temperature and environment	Wall material	Use straw bales to provide insulation from cold concrete

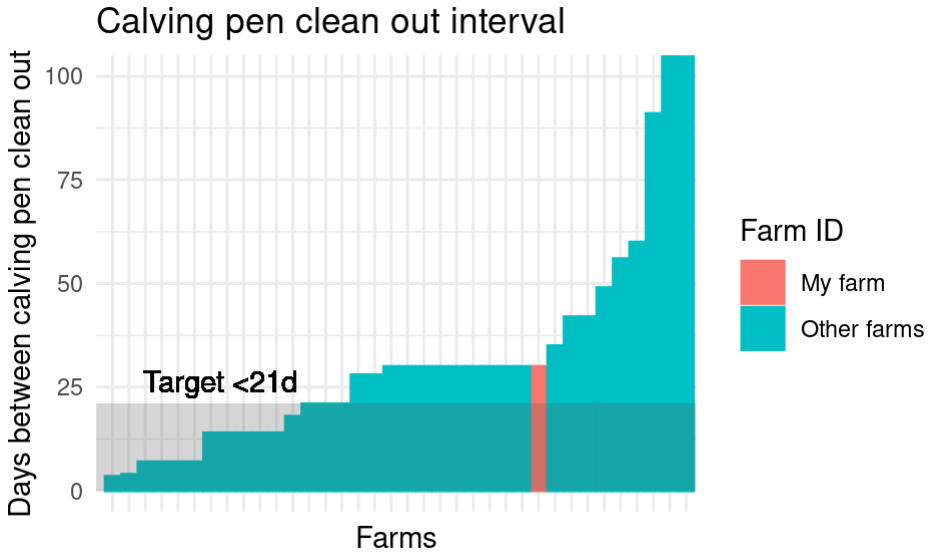
Calving pen use

You currently use a dedicated calving pen for 100% of calvings. Calving in a dedicated calving pen should improve hygiene of the calves at birth. The following graph shows your calving pen usage compared with other farms.



Hygiene in the calving pen is essential and can have a big impact on calf health. You currently clean out your calving pen every 30days on average.

The following graph provides a benchmark of how often your farm cleans out the calving pen compared with other farms.

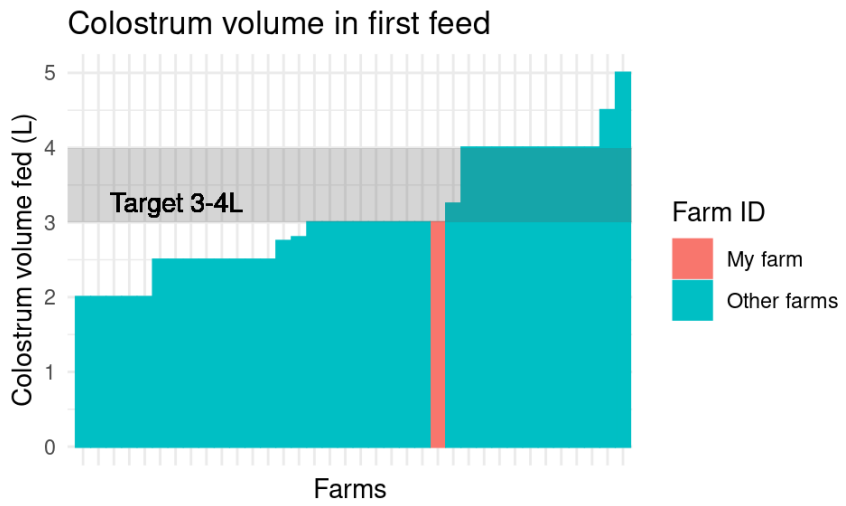


Calving pen recommendations

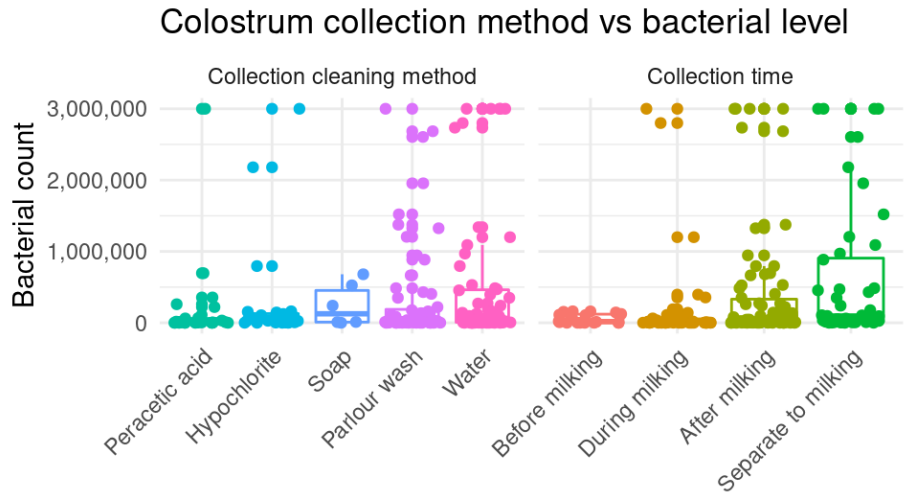
- Calving pen usage: Keep at this rate
- Cleaning interval: Clean out calving pen as frequently as possible, and at least every 21d

Colostrum feeding

You currently feed 3L of colostrum at first feeding, which is always within 6hours of birth. Colostrum feeding is essential for achieving passive transfer of immunity and should be fed as soon as possible after birth. The following graph shows your colostrum feeding volume compared with other farms.



Your colostrum collection is currently before milking, and cleaning of colostrum collection equipment is with parlour wash. The hygiene of colostrum is essential to ensure calves get good immunity from their colostrum, as well as reducing the chance of infection during the first feed. Below is a graph showing colostrum contamination levels of samples in relation to when they were collected and how the equipment was cleaned.



Colostrum recommendations

- Volume: Keep at this rate
- Timing: Keep at this rate
- Collection time: This is a great time to avoid contamination of colostrum, continue at this time
- Collection cleaning: This is NOT an appropriate cleaning product. Use hypochlorite, peracetic acid or soap after every use

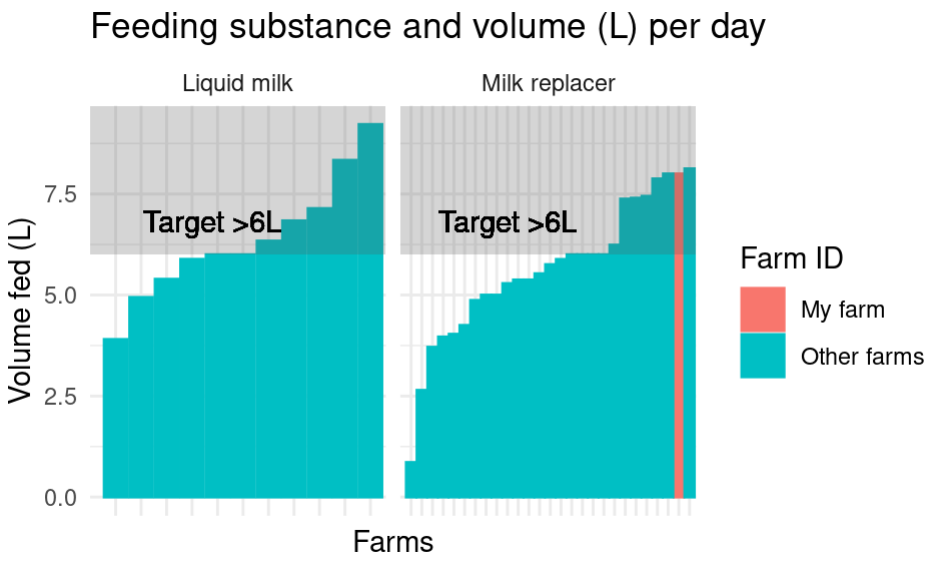
Milk feeding

Your current feeding plan is to feed 8 litres of milk replacer powder per day, in a twice daily feeding plan. Correct milk feeding of calves is crucial not only to achieving good daily liveweight gains, but also in the reducing the risk of disease and mortality.

Research has shown that calves will voluntarily drink at least 10 litres of milk per day and increasing milk feeding volume can improve growth rates as well as decreased risk of disease and mortality. As calves have developed to suckle regularly from their mothers, it is important to feed several times throughout the day possible (i.e., with automatic feeders), with an absolute minimum of two feeds per day if being fed by hand. There is some evidence that feeding liquid milk might reduce the risk of disease and may be nutritionally superior to milk replacer powder.
If you wish to feed powder, ensure that the powder weight is WEIGHED out each time using kitchen scales rather than a jug, to reduce inconsistent concentrations. The AHDB milk replacer calculator can be used to calculate an appropriate volume and concentration of milk to be fed for different environmental temperatures.

If you choose to feed liquid milk rather than powder it must NOT be antibiotic waste milk and must ONLY come from animals that are disease negative, particularly Johnes and Mycoplasma. This should be discussed with your veterinarian before altering.

Below is a benchmark of your feeding per day compared with other farms.



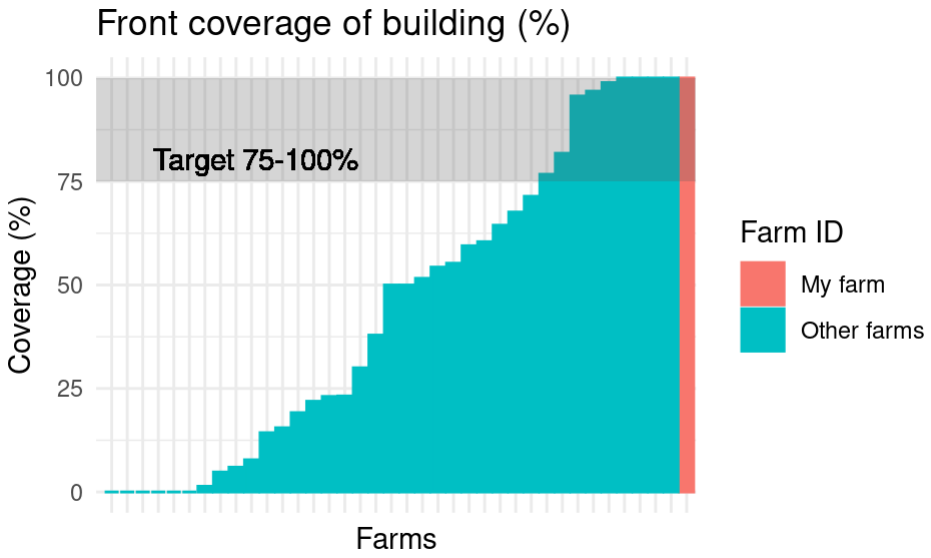
Milk feeding recommendations

- Volume: Good level of feeding
- Frequency: Consider increasing feeding frequency or using auto or ad lib feeding
- Substance: Consider feeding liquid milk. NOT antibiotic waste milk and ONLY from animals with known disease negative status (i.e. Johnes, Mycoplasma etc) in accordance with advice from your veterinarian.

Temperature and environment

Providing warm and clean shelter for preweaned calves is essential to maintaining health and welfare. Preweaned calves do not generate the same heat as older cattle and require a warmer environment than adults. Preweaning calves on your farm currently have 100% coverage with a roof, and 100% coverage at the front of the building for wind. The building walls are made from concrete, and there are straw bale shelters within shed.

Below is a graph showing what % of the front of your preweaning calf building is covered compared with other farms.



Calves that are within the first month of life are likely to be too cold if the environmental temperature is colder than 15 degrees Celsius. Research has shown that calf buildings in the UK are often very cold over winter and consequently calves are at a greater risk of disease, death and poor performance.

Infrared heat lamps do not heat the building but do provide heat to animals nearby. A 1kW heat lamp is only around £100 to purchase and costs around £100 per month to run (based on 13p/KwH). Heat lamps can be shared between two calves if in individual/dual pens, or between 2-4 calves if in group housing, and can be used in cold conditions for calves under one month of age.

Extreme caution is required when using heat lamps to avoid the risk of fire. Ensure lamps are securely fastened in accordance with manufacturer instructions and use metal chain fixings to solid structures.

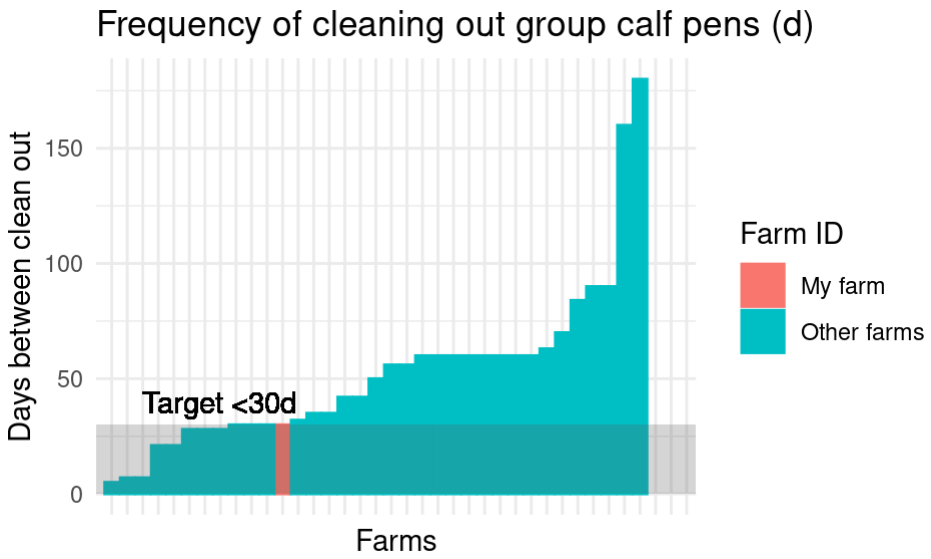
Temperature and environment recommendations

- Roof protection: Optimal roof coverage
- Wind protection: Good level of protection
- Wall material: Use straw bales to provide insulation from cold concrete
- Calf shelters: Good shelter for calves within shed
- Heating: Consider using active heating over winter. For example, patio heaters or heat lamps.

General management

Regularly cleaning out calf buildings, and providing clean dry bedding is an excellent method of reducing disease risk and ensuring optimal growth. Group calf housing is cleaned out every 30 days and is bedded up every at least daily.

Below is a graph showing how often you currently clean out groups of preweaned calves compared with other farms. A good target is to clean out completely at least every 30d.



Sharing airspace with older animals (i.e., postweaning) is a risk factor for spreading bacteria and viruses that might cause pneumonia. On average preweaned calves share building airspace with 0 weaned animals. The target is to have zero weaned animals sharing airspace with preweaned animals.

Disbudding can be stressful and painful for calves and should be avoided when possible, by using polled breeds. If disbudding must be performed, then it should be performed as early as possible with the use of both local anaesthetic and a non-steroidal anti-inflammatory (NSAID). You currently disbud calves at 4 weeks of age. A good target is to disbud as soon as possible, or at the latest within 4 weeks of age.

General recommendations

- Clean out: Good level of cleaning
- Bedding frequency: Good frequency of bedding. Clean, dry bedding should be added to a height where calves' legs are not visible when they are lying down
- Bedding quantity: Increase bedding quantity to a level where calves' legs cannot be seen when lying down
- Airspace: No shared airspace with weaned animals decreases the chance of passing on disease
- Disbudding: Continue disbudding at this age. Consider polled breeds to avoid the need for disbudding and make sure to use an NSAID as well as local anaesthetic

Agreed recommendations

The following recommendations have been discussed and agreed to be implemented to improve health and productivity of preweaned calves. Please record recommendations to be implemented.

Area	Outcome	Recommendation	Selected
Calving pen	Calving pen clean out	Clean out calving pen as frequently as possible, and at least every 21d	
Colostrum	Colostrum collection cleaning	This is NOT an appropriate cleaning product. Use hypochlorite, peracetic acid or soap after every use	
Feeding	Feeding frequency	Consider increasing feeding frequency or using auto or ad lib feeding	
Feeding	Milk feeding	Consider feeding liquid milk. NOT antibiotic waste milk and ONLY from animals with known disease negative status (i.e. Johnes, Mycoplasma etc) in accordance with advice from your veterinarian.	
General management	Bedding quantity	Increase bedding quantity to a level where calves' legs cannot be seen when lying down	
General management	Routine testing	Consider increased monitoring to regularly monitoring calves for immune transfer as well as colostrum quality (using a brix refractometer) and hygiene (using bacteriology)	
Temperature and environment	Active heating	Consider using active heating over winter. For example patio heaters or heat lamps.	
Temperature and environment	Wall material	Use straw bales to provide insulation from cold concrete	

Automated calf health report code

This section contains the code utilised to write the custom report demonstrated in the previous section.

````
---
title: "Calf health report"
output:
    html_document:
      number_sections: false
      fig_width: 5
      fig_height: 3
      df_print: paged

params:
  table: NA
  top_rec: NA
---

```{r  global_options, echo=FALSE}
knitr::opts_chunk$set(error = FALSE, message = FALSE,
warning = FALSE)
```

```{r, echo=FALSE, eval=FALSE}
htmltools::img(src = knitr::image_uri("nottingham.jpg"),
               alt = 'logo',
               style = 'position:absolute; top:0; right:0; padding:10px; width:200px')
```

```{r echo=FALSE}
library(tidyverse)
library(kableExtra)

df <- params$table
top_rec <- params$top_rec
```

```{r echo=FALSE}
colostrum_bact <- read_csv("colostrum_bacteriology.csv")%>%
      mutate(farm_id = as.factor(farm_id),
         tbc_tvs = as.character(tbc_tvs),
         tbc_tvs = str_remove_all(tbc_tvs, "[>,-]"),
         tbc_tvs = as.numeric(tbc_tvs),
         tbc_tvs = case_when(tbc_tvs>3000000 ~ 3000000,
                             TRUE ~ tbc_tvs),
         coliform_count = as.character(coliform_count),
         coliform_count = str_remove_all(coliform_count, "[>,-]"),
         coliform_count = as.numeric(coliform_count),
         coliform_count = case_when(coliform_count>3000000 ~ 3000000,
                             TRUE ~ coliform_count),
         date_collected = dmy(date_collected),
         date_tested = dmy(date_tested),
         arrival_temp = as.character(arrival_temp),
         arrival_temp = case_when(arrival_temp=="ambiant" ~ "21",
                                  TRUE ~ arrival_temp),
         arrival_temp = as.numeric(arrival_temp)
        ) %>%
  mutate_if(is.character, as.factor)
```

```{r echo=FALSE}
benchmark_data <- read_csv("export_data.csv") %>%
  mutate(farm_id = as.character(farm_id),
         mean_freq_per_day = as.character(mean_freq_per_day),
         group1_mean_wall_temp = as.character(group1_mean_wall_temp),
         group_bedding_added_d = case_when(group_bedding_added_d <= 1 ~ "At least
daily",
                                group_bedding_added_d > 1 ~ "Less than daily",
                                is.na(group_bedding_added_d) ~ "Not done"))
```

```{r echo=FALSE}
df <- df %>%
  mutate(farm_id = as.character("my_farm"),
         group1_mean_wall_temp = as.character(group1_mean_wall_temp),
         group_bedding_added_d) %>%
````
````

```
  mutate_at(c("calving_pen_clean_interval_d",
              "colostrum_quantity",
              "disbudding_age_wk",
              "group_cleaning_freq_d",
              "group1_rain_shelter_pct",
              "mean_solid_front_pct",
              "longest_colostrum_within_hr",
              "mean_fed_per_day",
              "percent_use_calving_pen_mean"), as.numeric) %>%
  bind_rows(benchmark_data) %>%
  select(farm_id, everything()) %>%
  mutate(colostrum_colection_time = case_when(is.na(colostrum_colection_time) ~ "Not
done",
                                              colostrum_colection_time == "After
Milking" ~ "After milking",
                                              colostrum_colection_time == "Before
Milking" ~ "Before milking",
                                    colostrum_colection_time == "Before or after
milking" ~ "After milking",
                                    colostrum_colection_time == "During milking"
~ "During milking",
                                    colostrum_colection_time == "During Milking"
~ "During milking",
                                    colostrum_colection_time == "Separate to
milking" ~ "Separate to milking",
                                    colostrum_colection_time == "In the parlour
before milking" ~ "Before milking",
                                    colostrum_colection_time == "In the parlour
during milking" ~ "During milking",
                                    colostrum_colection_time == "In the parlour
after milking" ~ "After milking",
                                              TRUE ~ colostrum_colection_time),
            colostrum_collection_cleaned = case_when(colostrum_collection_cleaned ==
"After milking" |
                                                       colostrum_collection_cleaned
== "Hot wash" |
                                                       colostrum_collection_cleaned
== "Hot water" |
                                                       colostrum_collection_cleaned
== "Water" |
                                                       colostrum_collection_cleaned
== "Hot water scrub" ~ "Water",
                                                       colostrum_collection_cleaned
== "Parlour wash" |
                                                      colostrum_collection_cleaned
== "Rinse, parlour wash and hot soap scrub once per day" ~ "Parlour wash",
                                                     colostrum_collection_cleaned ==
"Hot soap scrub" ~ "Soap",
                                                       colostrum_collection_cleaned
== "Hot water and hypochlorite" |
                                                       colostrum_collection_cleaned
== "Hypochlorite" ~"Hypochlorite",
                                                       colostrum_collection_cleaned
== "Peracetic acid" ~ "Peracetic acid",

is.na(colostrum_collection_cleaned) ~ "Not done",
                                                     TRUE ~
colostrum_collection_cleaned)) %>%
  mutate(my_farm = case_when(farm_id == "my_farm" ~ "My farm",
                             farm_id != "my_farm" ~ "Other farms"),
         feed_liquid_milk = case_when(is.na(main_feed) ~ feed_liquid_milk,
                                      main_feed == "Milk replacer powder" ~ "Not
liquid",
                                      main_feed == "Liquid milk" ~ "Liquid"),
         feed_liquid_milk = case_when(feed_liquid_milk == "Liquid" ~ "Liquid milk",
                                      feed_liquid_milk == "Not liquid" ~ "Milk
replacer"))

pull_func <- function(target){
  df %>%
    filter(my_farm == "My farm") %>%
    select(target) %>%
    pull()
}

interested_in <- df %>%
```

```
    filter(my_farm == "My farm") %>%
    select(interested_in) %>%
  mutate(interested_in = str_replace(interested_in, "scour", "Scour"),
         interested_in = str_replace(interested_in, "pneum", "Pneumonia"),
         interested_in = str_replace(interested_in, "mort", "Mortality"),
         interested_in = str_replace(interested_in, "dlwg", "Growth rates"),
         interested_in = tolower(interested_in))
```

\
\

This calf health report is aimed to provide guidance in reducing the risk of morbidity and mortality and improving health and welfare.

This report is intended as guidance only and it is essential that any potential interventions are discussed with your farms' veterinarian before implementation. Benchmarking data is based on a study of 60 dairy farms across Great Britain in 2019.

`r paste("You have said you are most interested in improving ", interested_in, ".", sep = "")`

To improve these areas, the most important areas to focus on improving are as follows.

Top recommendations:
```{r echo=FALSE}
top_rec %>%
  kable() %>%
  kable_styling()
```

```{r echo=FALSE, eval=FALSE}
## Top Recommendations
 * **`r paste(top_rec[1,1])`**
    + `r paste(top_rec[1,2])`
 * **`r paste(top_rec[2,1])`**
    + `r paste(top_rec[2,2])`
 * **`r paste(top_rec[3,1])`**
    + `r paste(top_rec[3,2])`
 * **`r paste(top_rec[4,1])`**
    + `r paste(top_rec[4,2])`
 * **`r paste(top_rec[5,1])`**
    + `r paste(top_rec[5,2])`
 * **`r paste(top_rec[6,1])`**
    + `r paste(top_rec[6,2])`
```
<P style="page-break-before: always">
Calving pen use
\
`r paste("You currently use a dedicated calving pen for ", pull_func("percent_use_calving_pen_mean") %>% tolower(), "% of calvings.", sep = "")`

Calving in a dedicated calving pen should improve hygiene of the calves at birth.

The following graph shows your calving pen usage compared with other farms.

```{r echo=FALSE}
df  %>%
  ggplot(aes(x=reorder(farm_id, percent_use_calving_pen_mean),
y=percent_use_calving_pen_mean, colour=my_farm, fill=my_farm))+
  geom_bar(stat="identity")+
    annotate("rect", xmin = 0, xmax = Inf, ymin = 95, ymax = 100,
             alpha = .25)+
  geom_text(aes(1,95,label = "Target 95-100%", vjust = -0.5, hjust = -0.2),
colour="black")+
  labs(title = "Use of calving pen",
       x="Farms",
       y="Percent use of calving pen (%)",
       fill="Farm ID",
       colour="Farm ID")+
  theme_minimal()+
  theme(axis.text.x = element_blank())
```

Hygiene in the calving pen is essential and can have a big impact on calf health.

```
`r paste("You currently clean out your calving pen every ",
pull_func("calving_pen_clean_interval_d"), "days on average.", sep = "")`

The following graph provides a benchmark of how often your farm cleans out the
calving pen compared with other farms.

```{r echo=FALSE}
target <- 21

df %>%
  filter(!is.na(calving_pen_clean_interval_d)) %>%
  ggplot(aes(x=reorder(farm_id, calving_pen_clean_interval_d),
y=calving_pen_clean_interval_d, colour=my_farm, fill=my_farm))+
  geom_bar(stat="identity")+
  annotate("rect", xmin = 0, xmax = Inf, ymin = 0, ymax = 21,
           alpha = .25)+
  geom_text(aes(1,21,label = "Target <21d", vjust = -0.5, hjust = -0.2),
colour="black")+
  labs(title = "Calving pen clean out interval",
       x="Farms",
       y="Days between calving pen clean out",
       fill="Farm ID",
       colour="Farm ID")+
  theme_minimal()+
  theme(axis.text.x = element_blank())+
  coord_cartesian(ylim = c(0, 100))
```

Calving pen recommendations
 * **`r paste("Calving pen usage: ",
pull_func("advice_percent_use_calving_pen_mean"), sep = "")`**
 * **`r paste("Cleaning interval: ",
pull_func("advice_calving_pen_clean_interval_d"))`**

<P style="page-break-before: always">
Colostrum feeding

`r paste("You currently feed ", pull_func("colostrum_quantity"), "L of colostrum at
first feeding, which is always within ", pull_func("longest_colostrum_within_hr"),
"hours of birth.", sep = "")`

Colostrum feeding is essential for acheiving passive transfer of immunity, and should
be fed as soon as possible after birth.

The following graph shows your colostrum feeding volume compared with other farms.

```{r echo=FALSE}
df %>%
  filter(!is.na(colostrum_quantity)) %>%
  ggplot()+
  geom_bar(aes(x=reorder(farm_id, colostrum_quantity), y=colostrum_quantity,
colour=my_farm, fill=my_farm),stat="identity")+
#  geom_rect(aes(ymin=3, ymax=4, xmin=0, xmax=Inf, alpha=0.1))+
  annotate("rect", xmin = 0, xmax = Inf, ymin = 3, ymax = 4,
           alpha = .25)+
  geom_text(aes(1,3,label = "Target 3-4L", vjust = -0.5, hjust=-0.2),
colour="black")+
  labs(title = "Colostrum volume in first feed",
       x="Farms",
       y="Colostrum volume fed (L)",
       fill="Farm ID",
       colour="Farm ID")+
  theme_minimal()+
  theme(axis.text.x = element_blank())
```


`r paste("You colostrum collection is currently ",
pull_func("colostrum_colection_time") %>% tolower(), ", and cleaning of colostrum
collection equipment is with ", pull_func("colostrum_collection_cleaned") %>%
tolower(), ".", sep = "")`

The hygiene of colostrum is essential to ensure calves get good immunity from their
colostrum, as well as reducing the chance of infection during the first feed.
```

Below is a graph showing colostrum contamination levels of samples in relation to when they were collected and how the equipment was cleaned.

```{r echo=FALSE}
colostrum_bact %>%
  left_join(df) %>%
   filter(colostrum_colection_time != "Not done",
         !is.na(colostrum_colection_time)) %>%
  filter(colostrum_collection_cleaned != "Not done",
         !is.na(colostrum_collection_cleaned)) %>%
  gather(key, value, colostrum_collection_cleaned, colostrum_colection_time) %>%
   mutate(value = fct_relevel(value,
                              "Before milking",
                              "During milking",
                              "After milking",
                              "Separate to milking",
                              "Peracetic acid",
                              "Hypochlorite",
                              "Soap",
                              "Parlour wash",
                              "Water"),
          key = case_when(key == "colostrum_colection_time" ~ "Collection time",
                          key == "colostrum_collection_cleaned" ~ "Collection
cleaning method")) %>%
  select(key, value, tbc_tvs) %>%
  rename(`Total bacterial count` = tbc_tvs) %>%
  ggplot()+
  geom_boxplot(aes(x = value, y=`Total bacterial count`, colour = value))+
  geom_jitter(aes(x = value, y=`Total bacterial count`, colour = value))+
  theme(axis.text.x = element_text(angle = 45, hjust = 1))+
  labs(title = "Colostrum collection method vs bacterial level",
       y = "Bacterial count",
       x = "")+
  scale_y_continuous(labels = scales::comma)+
  facet_wrap(~key, scales="free_x")+
  theme_minimal()+
  theme(legend.position = "none")+
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

Colostrum recommendations
 * **`r paste("Volume: ", pull_func("advice_colostrum_quantity"), sep = "")`**
 * **`r paste("Timing: ", pull_func("advice_longest_colostrum_within_hr"), sep =
"")`**
 * **`r paste("Collection time: ", pull_func("advice_colostrum_colection_time"), sep
= "")`**
 * **`r paste("Collection cleaning: ",
pull_func("advice_colostrum_collection_cleaned"), sep = "")`**

<P style="page-break-before: always">
Milk feeding

`r paste("Your current feeding plan is to feed ", pull_func("mean_fed_per_day"), "
litres of ", pull_func("main_feed") %>% tolower(), " per day, in a " ,
pull_func("mean_freq_per_day") %>% tolower(), " feeding plan." , sep = "")`

Correct milk feeding of calves is crucial not only to acheiving good daily liveweight gains, but also in the reducing the risk of disease and mortality.

Research has shown that calves will voluntarily drink at least 10 litres of milk per day, and increasing milk feeding volume can improve growth rates as well as decrease risk of disease and mortality. As calves have developed to suckle regularly from their mothers, it is important to feed several times throughout the day possible (i.e. with automatic feeders), with an absolute minimum of two feeds per day if being fed by hand. There is some evidence that feeding liquid milk might reduce the risk of disease, and may be nutritionally superior to milk replacer powder.

If you wish to feed powder, ensure that the powder weight is WEIGHED out each time using kitchen scales rather than a jug, to reduce inconsistent concentrations. The [AHDB milk replacer calculator](http://herdhealth.shinyapps.io/calfhealth/) can be used to calculate an appropriate volume and concentration of milk to be fed for different environmental temperatures.

If you choose to feed liquid milk rather than powder it must NOT be antibiotic waste milk, and must ONLY come from animals that are disease negative, particularly Johnes and Mycoplasma. This should be discussed with your veterinarian before altering.

Below is a benchmark of your feeding per day compared with other farms.

```{r echo=FALSE}
target <- 6

df %>%
  ggplot(aes(x=reorder(farm_id, mean_fed_per_day), y=mean_fed_per_day,
colour=my_farm, fill=my_farm))+
  geom_bar(stat="identity")+
  annotate("rect", xmin = 0, xmax = Inf, ymin = 6, ymax = Inf,
           alpha = .25)+
  geom_text(aes(x = 1, y = 6, label = "Target >6L", vjust = -1, hjust = -0.2),
colour="black")+
  labs(title = "Feeding substance and volume (L) per day",
       x="Farms",
       y="Volume fed (L)",
       fill="Farm ID",
       colour="Farm ID")+
  theme_minimal()+
  theme(axis.text.x = element_blank())+
  facet_wrap(~feed_liquid_milk, scales="free_x")
```

Milk feeding recommendations
* **`r paste("Volume: ", pull_func("advice_mean_fed_per_day"), sep = "")`**
* **`r paste("Frequency: ", pull_func("advice_mean_freq_per_day"), sep = "")`**
* **`r paste("Substance: ", pull_func("advice_main_feed"), sep = "")`**

<P style="page-break-before: always">
Temperature and environment

Providing warm and clean shelter for preweaned calves is essential to maintaining health and welfare. Preweaned calves do not generate the same heat as older cattle, and require a warmer environment than adults.

`r paste("Preweaning calves on your farm currently have ",
pull_func("group1_rain_shelter_pct"), "% coverage with a roof, and ",
pull_func("mean_solid_front_pct"), "% coverage at the front of the building for wind.", sep = "")`

`r paste("The building walls are made from ", pull_func("group1_mean_wall_temp") %>% tolower(), ", and there are ", pull_func("calf_shelters") %>% tolower(), ".", sep = "")`

Below is a graph showing what % of the front of your preweaning calf building is covered compared with other farms.

```{r echo=FALSE}
df %>%
  ggplot(aes(x=reorder(farm_id, mean_solid_front_pct), y=mean_solid_front_pct,
colour=my_farm, fill=my_farm))+
  geom_bar(stat="identity")+
  annotate("rect", xmin = 0, xmax = Inf, ymin = 75, ymax = 100,
           alpha = .25)+
  geom_text(aes(1,75,label = "Target 75-100%", vjust = -0.5, hjust = -0.2),
colour="black")+
  labs(title = "Front coverage of building (%)",
       x="Farms",
       y="Coverage (%)",
       fill="Farm ID",
       colour="Farm ID")+
  theme_minimal()+
  theme(axis.text.x = element_blank())
```

Calves that are within the first month of life are likely to be too cold if the environmental temperature is colder than 15 degrees celsius. Research has shown that calf buildings in the UK are often very cold over winter and consequently calves are at a greater risk of disease, death and poor performance.

Infrared heat lamps do not heat the building, but do provide heat to animals nearby. A 250W heat lamp is only around £25 to purchase and costs around £20-25 per month to run (based on 13p/KwH). Heat lamps can be shared between two calves if in individual/dual pens, or between 2-4 calves if in group housing, and can be used in cold conditions for calves under one month of age.

```
Extreme caution is required when using heat lamps to avoid the risk of fire. Ensure
lamps are securely fastened in accordance with manufacturer instructions and use
metal chain fixings to solid structures.

### Temperature and environment recommendations
* **`r paste("Roof protection: ", pull_func("advice_group1_rain_shelter_pct"), sep =
"")`**
* **`r paste("Wind protection: ", pull_func("advice_mean_solid_front_pct"), sep =
"")`**
* **`r paste("Wall material: ", pull_func("advice_group1_mean_wall_temp"), sep =
"")`**
* **`r paste("Calf shelters: ", pull_func("advice_calf_shelters"), sep = "")`**
* **`r paste("Heating: ", pull_func("advice_active_heating"), sep = "")`**

<P style="page-break-before: always">
# General management

Regularly cleaning out calf buildings, and providing clean dry bedding is an
excellent method of reducing disease risk and ensuring optimal growth.

`r paste("Group calf housing is cleaned out every ",
pull_func("group_cleaning_freq_d"), " days, and is bedded up every ",
pull_func("group_bedding_added_d") %>% tolower(), ".", sep = "")`

Below is a graph showing how often you currently clean out groups of preweaned calves
compared with other farms. A good target is to clean out completely at least every
30d.

```{r echo=FALSE}
target <- 30

df %>%
  ggplot(aes(x=reorder(farm_id, group_cleaning_freq_d), y=group_cleaning_freq_d,
colour=my_farm, fill=my_farm))+
  geom_bar(stat="identity")+
  annotate("rect", xmin = 0, xmax = Inf, ymin = 0, ymax = target,
           alpha = .25)+
  geom_text(aes(1,30,label = "Target <30d", vjust = -0.5, hjust = -0.2),
colour="black")+
  labs(title = "Frequency of cleaning out group calf pens (d)",
       x="Farms",
       y="Days between clean out",
       fill="Farm ID",
       colour="Farm ID")+
  theme_minimal()+
  theme(axis.text.x = element_blank())
```

Sharing airspace with older animals (i.e. postweaning) is a risk factor for spreading
bacteria and viruses that might cause pneumonia.

`r paste("On average preweaned calves share building airspace with ",
pull_func("mean_weaned_shed") %>% tolower(), " weaned animals.", sep = "")`

The target is to have zero weaned animals sharing airspace with preweaned animals.

Disbudding can be stressful and painful for calves and should be avoided when
possible by using polled breeds. If disbudding must be performed then it should be
performed as early as possible with the use of both local anaesthetic and a non-
steroidal anti-inflammatory (NSAID).
`r paste("You currently disbud calves at ", pull_func("disbudding_age_wk"), " weeks
of age. A good target is to disbud as soon as possible, or at the latest within 4
weeks of age.", sep = "")`

General recommendations
* **`r paste("Clean out: ", pull_func("advice_group_cleaning_freq_d"), sep = "")`**
* **`r paste("Bedding frequency: ", pull_func("advice_group_bedding_added_d"), sep =
"")`**
* **`r paste("Bedding quantity: ", pull_func("advice_nesting"), sep = "")`**
* **`r paste("Airspace: ", pull_func("advice_mean_weaned_shed"), sep = "")`**
* **`r paste("Disbudding: ", pull_func("advice_disbudding_age_wk"), sep = "")`**

<P style="page-break-before: always">
Agreed recommendations
```

The following recommendations have been discussed and agreed to be implemented to improve health and productivity of preweaned calves.

Please record the date each agreed recommendation of the plan was implemented. If the agreed recommendation was stopped for any reason please record this in the Date Stopped column (leave blank if ongoing)

```{r echo=FALSE}
top_rec %>%
  mutate("Selected" = " ",
         "Date started" = " ",
         "Date if stopped" = " ") %>%
  kable() %>%
  kable_styling()
```

Appendix iii

The following section provides a selection of modules to demonstrate the *stabiliser* R package created during the course of this thesis.

stabiliser R package

```
#' stabilise
#'
#' @name stabilise
#'
#' @description Function to calculate stability of variables' association with an
outcome for a given model over a number of bootstrap repeats
#'
#' @param data a dataframe containing an outcome variable to be permuted
#' @param outcome the outcome as a string (i.e. "y")
#' @param boot_reps the number of bootstrap samples
#' @param permutations the number of times to be permuted per repeat
#' @param perm_boot_reps the number of times to repeat each set of permutations
#'
#' @import rsample
#' @import dplyr
#' @importFrom purrr map
#'
#' @export
#'

utils::globalVariables(c("models"))

stabilise <- function(data, outcome, boot_reps, permutations, perm_boot_reps, models)
{

  boot_data = boot_sample(data=data, boot_reps=boot_reps)
  perm_data = perm_sample(data=data, outcome=outcome, permutations = permutations,
perm_boot_reps = perm_boot_reps)

  output <- models %>%
    map(., ~ perm_stab(
      data = data,
      boot_data = boot_data,
      perm_data = perm_data,
      outcome = outcome,
      boot_reps = boot_reps,
      permutations = permutations,
      perm_boot_reps = perm_boot_reps,
      model_name = .
    ))

  names(output) <- models

  output
}
```

```r
#' perm_stab
#'
#' @name perm_stab
#'
#' @description Main function to call both permutation and bootstrapping functions;
to be looped over multiple models selected by the user.
#'
#' @importFrom tidyr nest
#'

utils::globalVariables(c('data', 'outcome', 'boot_reps', 'permutations',
'perm_boot_reps', 'model_name'))

perm_stab <- function(data, boot_data, perm_data, outcome, boot_reps, permutations,
perm_boot_reps, model_name) {
  selected_model <- model_selector(model_name)

  message("Permuting ", model_name, "...")
  perm_coefs <- perm_model(perm_data = perm_data, data = data, outcome = outcome,
permutations = permutations, perm_boot_reps = perm_boot_reps, selected_model =
selected_model)
  perm_thresh <- perm_summarise(permed_object = perm_coefs)
  message("Done")
  message("Stabilising ", model_name, "...")
  coefs <- boot_model(data = boot_data, outcome = outcome, boot_reps = boot_reps,
selected_model = selected_model)
  stability <- boot_summarise(booted_obj = coefs, data = data, boot_reps = boot_reps)
%>%
    mutate(stable = case_when(stability > perm_thresh ~ "*"))
  message("Done")

  list(
    "stability" = stability,
    "boot_coefs" = coefs %>%
      group_by(bootstrap) %>%
      nest() %>%
      rename(variables = data),
    "perm_thresh" = perm_thresh,
    "perm_coefs" = perm_coefs
    )
}
```

```r
#' boot_model
#'
#' @name boot_model
#'
#' @description Function to calculate stability of variables' association with an
outcome for a given model over a number of bootstrap repeats
#'
#' @param data a dataframe containing an outcome variable to be permuted
#' @param outcome the outcome as a string (i.e. "y")
#' @param boot_reps the number of bootstrap samples
#' @param model the model to be used (i.e. model_mbic)
#'
#' @import rsample
#' @import dplyr
#' @importFrom purrr map_df
#' @importFrom utils globalVariables
#' @importFrom tidyr replace_na
#' @importFrom stats quantile
#'

utils::globalVariables(c(".", "variable", "stability", "estimate", "quantile",
"prop_one_side", "bootstrap_p"))

boot_sample <- function(data, boot_reps) {
  rsample::bootstraps(data, boot_reps)
}

boot_model <- function(data, outcome, boot_reps, selected_model) {
  data %>%
    map_df(.x = .$splits, .f = ~ as.data.frame(.) %>% selected_model(., outcome =
outcome), .id = "bootstrap")
}

boot_summarise <- function(booted_obj, data, boot_reps){
  booted_obj %>%
    group_by(variable) %>%
    summarise(
      mean_coefficient = mean(estimate, na.rm = TRUE),
      ci_lower = quantile(estimate, 0.025, na.rm = TRUE),
      ci_upper = quantile(estimate, 0.975, na.rm = TRUE),
      prop_one_side = case_when(
        mean_coefficient > 0 ~ length(estimate[estimate > 0]),
        mean_coefficient < 0 ~ length(estimate[estimate < 0])
      ),
      bootstrap_p = 1 - (prop_one_side / length(estimate)),
      stability = (n() / boot_reps) * 100
    ) %>%
    select(-prop_one_side) %>%
    right_join(tibble(variable = colnames(data)), by = "variable") %>%
    replace_na(list(
      stability = 0
    )) %>%
    arrange(desc(stability))
}
```

```r
#' model_enet
#'
#' @name model_enet
#'
#' @description Function to model elastic net selection process on a given dataframe
#'
#' @param data a dataframe containing an outcome variable to be permuted (usually
coming from nested bootstrap data)
#' @param outcome the outcome as a string (i.e. "y")
#'
#' @import glmnet
#' @import dplyr
#' @import broom
#' @importFrom stats coef
#' @importFrom utils globalVariables
#'

utils::globalVariables(c(".", "variable", "estimate", "value"))

model_enet <- function(data, outcome) {
  y_temp <- data %>%
    select(all_of(outcome)) %>%
    as.matrix()

  x_temp <- data %>%
    select(-all_of(outcome)) %>%
    as.matrix()

  fit_lasso <- cv.glmnet(x=x_temp, y=y_temp, type.measure = "mae", nfolds = 10)

  coef(fit_lasso, s = "lambda.min") %>%
    broom::tidy() %>%
    rename(variable = row,
           estimate = value) %>%
    filter(variable != "(Intercept)") %>%
    select(variable, estimate)
}
```