Identification of on-farm recorded data for the prediction of disease in dairy cattle

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Dedicated to Papa

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Declaration

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

Grace Louise Smith
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Not to us, LORD, not to us but to your name be the glory,
because of your love and faithfulness
Psalm 115:1 (NLT)

Since from His bounty I receive
Such proofs of love divine,
Had I a thousand hearts to give,
Lord, they should all be Thine.
S. Stennet

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Abstract

Identification of cows at increased disease risk during the transition period is necessary to reduce the negative economic impact of disease and to improve animal welfare. Although timely identification of at-risk cows is a vital component of health management, it is challenging in modern dairy herds, where staff manage an increasing number of cattle. The consequent reduction in time available for individual animal observation has created a need for the development of decision support tools which facilitate individual cow monitoring. However, uncertainty exists as to which measurable traits best reflect cow health status, especially in the dry and transition periods where little monitoring of individual cows is performed. Therefore, the objectives of this project were 1) to quantify the effect of early lactation disease on productivity 2) to identify variables of routinely recorded herd data which could be used for disease prediction or as risk factors for disease and 3) to assess the feasibility of using such indicators in predictive disease modelling.

Retrospective analyses were performed on 482 cow-lactations from the Langhill herd of Holstein cattle. Cow-lactations were assigned to 1 of 4 health groups based on disease incidence in the first 30 days of lactation. These groups were no clinical disease (NCD; n = 335, reproductive (REP; n = 77) (which included cases of retained placenta and metritis), subclinical mastitis (SCM; n = 53) (determined by somatic cell counts) and metabolic (MET; n = 17) (which included cases of displaced abomasum, ketosis, hypomagnesaemia and hypocalcaemia). The data were analysed using descriptive statistics, mixed models, and generalised linear mixed models, with a logit link, in SAS 9.3 and GenStat 16.

There were significant differences in average milk yield between health groups throughout lactation. In the first 30 days of lactation, NCD cows had significantly higher ($p<0.01$) daily milk yield than either REP, SCM or MET cows. Days to first observed heat and first service were significantly higher in MET cows than all other groups ($p<0.01$) and was extended by 27 days compared to NCD cows. No difference existed between services per conception or calving interval across all groups however the 100 day in-calf rate was reduced amongst cows with disease compared to cows without disease. Preceding disease, milk yield at dry-off and the ratio of energy corrected milk to body energy content were found to be significantly different between health groups; both measures were significantly higher in SCM cows compared to REP and MET cows. Additionally, in the first 15 days of the dry period preceding disease diagnoses, REP cows had a significantly (p=0.02) greater rate of change in body energy content than NCD cows; -18.3±7.44 MJ per day vs. 0.6±5.11 MJ per day, respectively. Overall change in body energy content between dry off and calving was significantly greater (p<0.001) in REP cows than both NCD and SCM cows.

The predictive ability of candidate indicators identified as being significantly different between health groups was assessed using further statistical analysis. The distribution of each candidate indicator was investigated before Pearson and Spearman correlation tests were used to quantify the relationships between indicators. Single candidate models, employing generalised linear mixed modelling with random effect for cow, were used to test the effect of each candidate indicator on each response measure (health group). Dry period length, change in live weight and body energy content across the dry period, condition score and body energy content at dry off and the rate of change in body energy content in the first 15 days of lactation were significant predictors (p<0.05) of reproductive disorders while the year of calving and live weight at calving were significant predictors (p<0.05) of subclinical mastitis when included in single candidate models.

Multivariate models for each of the disease response measures (REP, SCM and MET) were developed using combinations of the candidate indicators as explanatory variables. Despite some highly significant relationships between the candidate indicator variables and response measures, the multivariate models developed do not currently have potential to predict risk of disease at an acceptable level of accuracy, as very few significant effects were found. This can be explained by the large individual cow variance components and a low incidence of disease in the current data set.

Future research should focus on tracking candidate indicator data in individual cows with a view to establishing a baseline for each cow. This would allow each cow to be used as its own control, with deviations from the normal indicating potential disease challenge. This study has demonstrated that early lactation disease has both short- and long-term effects on productivity. Further, routine measures of herd data including body weight and body condition score, recorded in the dry period have been shown to be significantly different between cows of different disease status in the subsequent lactation.

This study has shown that disease in early lactation has serious consequences for the productivity of dairy cattle and has shown the potential for predicting the risk of disease in the transition period in dairy cows. However, further work is needed with larger datasets and in different herds to develop greater accuracy in prediction.

Lay summary

It is well documented that the transition from pregnancy to lactation represents a significant physiological challenge for the dairy cow. Such is the intensity of the physiological stress experienced by the cow during this transition that the risk of disease is significantly increased in early lactation. Inappropriate management of dairy cows in the 8 weeks prior to giving birth, when they are no longer producing milk, can exacerbate this stress and further increase the risk of disease. Common diseases associated with this period include mastitis, ketosis, left displaced abomasum, hypomagnesaemia, retained placenta and metritis. Each of these diseases have negative effects on both welfare and productivity, meaning that a reduction in disease incidence offers the opportunity to simultaneously improve animal welfare and profitability of dairy farming systems.

The identification of cows at increased risk of developing disease would be useful to make interventions to reduce this risk, or to strategically employ extra monitoring tools to identify cases of disease early and limit its severity.

Data collected over 8 years from one large herd of Holstein cows in south west Scotland was firstly used to quantify the effect that disease in early lactation has on measures of dairy cow productivity. Cows that remained healthy in the 30 days after giving birth produced significantly more milk than cows that developed subclinical mastitis, reproductive or metabolic disease. Additionally, cows that suffered from metabolic disease in the 30 days after calving took longer to resume reproductive activity after calving. The data was then analysed to identify measures which were significantly different between cows that remained healthy and those that developed disease before they became sick. Associations between changes in body weight and changes in body fat and future disease status were found; cows that developed reproductive disease were found to have lost body energy in the early stages of the dry period. The final stage of this study was to construct statistical models using the data to assess its ability to predict the risk of disease. However, even though there were differences in the data between cows that remained healthy and those that developed disease, it was not possible to develop predictive models that could identify cows at increased risk of disease. The range of measurements of body weight and energy content within the group of cows that remained healthy and within the groups of cows that developed different diseases, meant that the predictive model was not able to accurately discriminate between these animals.

This study has demonstrated that there is potential for data recorded on commercial dairy farms to be used in determining future health status, but further work is needed before this can incorporated into a practical tool to identify at risk cows on farm.

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List of abbreviations

2D	Two dimensional
3D	Three dimensional
AHDB	Agriculture & Horticulture Development Board
AI	Artificial insemination
AMS	Automatic milking system
ANOVA	Analysis of variance
BCS	Body condition score
BEC	Body energy content
BHB	Beta-hydroxybutyrate
BW	Body weight
C	Control genetic line
calvBCS	Body condition score at calving
calvBEC	Body energy content at calving (mega-joules)
calvBW	Body weight at calving (kilograms)
CS	Condition score
CSU	Condition score unit
DA	Displaced abomasum
DCAB	Dietary cation anion balance
DFH	Days to first heat
DFS	Days to first service
diffBCS	Body condition score at calving - body condition score at dry off
diffBEC	Body energy at calving - body energy at dry off (mega-joules)
diffBW	Body weight at calving - body weight at dry off (kilograms)
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
doBCS	Body condition score at dry off
doBEC	Body energy content at dry off (mega-joules)
doBW	Body weight content at dry off (kilograms)
dp	Days pregnant
ECM	Energy corrected milk yield (litres)
FAO	Food and Agriculture Organisation of the United Nations

FTIR	Fourier transform infrared
FPR	Fat:protein ratio
g	Gram
GLM	Generalised linear model
GLMM	Generalised linear mixed model
HF	High Forage
HFC	High Forage Control
HFS	High Forage Select
IMI	Intramammary infection
kg	Kilograms
LDA	Left displaced abomasum
LF	Low Forage
LFC	Low Forage Control
LFS	Low Forage Select
LSM	Least squares mean
MBER	Milk yield: body energy ratio
MEC	Milk electrical conductivity
mEq/kg	milliequivalent/kilogram
MET	Metabolic disorder
MIR	Mid infrared
MJ	mega-joules
mmol/L	millimol/litre
MYAcc	Milk yield acceleration
NCD	No clinical disease
NCGD	Neutral cellulase gammanase digestibility
NEB	Negative energy balance
NEFA	Non-esterified fatty acid
NRC	National Research Council
PLI	Profitable Lifetime Index
PTA	Predicted Transmitting Ability
REP	Reproductive disorder
ROS	Reactive oxidative species
RP	Retained placenta
S	Select genetic line

SARA	Subacute ruminal acidosis
s.e	Standard error
s.e.m	Standard error of the mean
SCC	Somatic cell count
SCK	Subclinical ketosis
SCM	Subclinical mastitis
SDCT	Selective dry cow therapy
slope15BCS	Slope of body condition score change in first 15 days of dry period
slope15BEC	Slope of body energy content change in first 15 days of dry period (mega-joules per day)
slope15BW	Slope of body weight change in first 15 days of dry period (kilograms per day)
SPC	Services per conception
SRUC	Scotland's Rural College
TMR	Total mixed ration
umol/L	micromol/litre

Chapter One

General Introduction and Literature Review

1.1 General Introduction

Global human population growth and increased per capita consumption of dairy products continue to drive demand for milk production (FAO, 2012). By 2030, milk consumption is projected to be 89.5kg per person per year; an increase of 21% since 1964, with the greatest growth in consumption forecasted to occur in developing countries such as India (Hobbs *et al*., 2016). Concurrent with the increased demand for dairy products has been a global increase in average milk yield per cow. In the United States, milk production per cow increased by 5977 kg between 1957 and 2007 (Van Raden, 2004). Similar trends have been seen in developing economies. Average annual milk yield per cow in China is forecast to be 3009 kg per year by 2025, a projected increase of 1021 kg from 2010 (Statista, 2011). In Europe, the average yield of Swedish dairy cows increased from 4200kg to 9000kg per year between 1957 and 2003, while the average yield of Holstein dairy cows in Austria increased from 5500kg to 8200kg per year between 1988 to 2007 (Oltenacu and Broom, 2010). In the United Kingdom, average milk yield per cow has increased from 4099 litres to 7916 litres per annum between 1975 and 2016 (Bate, 2016). This is equivalent to an average annual increase of 2.4% in production. It became of considerable concern that such increases in production were accompanied by declining fertility and an increase in the incidence of production disease, prior to the inclusion of health-related traits in breeding programmes (Ingvartsen *et al*., 2003).

Increased individual cow milk production has been achieved through a combination of improved management, nutrition, and genetic selection for increased productivity. Historically, production gains were primarily driven by advances in nutrition (Eastridge, 2006). Since the widespread adoption of artificial insemination and progeny testing in the mid-1980s, gains in milk production can much more be attributed to intense genetic selection for milk yield (Ingvartsen *et al*., 2003). To facilitate such gains, narrow breeding goals which focussed on milk yield and milk solids were implemented, to the exclusion of functional traits including health and fertility (Axelsson, 2013). Unfavourable genetic correlations between production and health traits indicate that the deterioration in health and fertility is primarily a consequence of intense genetic selection for increased milk yield, although the relationship between health and production is very complex (Oltenacu and Broom, 2010). By the end of the 20th century, emphasis shifted towards more balanced breeding goals with the inclusion of previously undervalued non-yield traits in breeding programmes (Miglior *et al*., 2017). The inclusion of health, welfare, and fitness traits in breeding programmes in the United Kingdom has led to considerable improvement; since its inclusion in the profitable lifetime index (£PLI) in 2007, fertility has started to rapidly recover and somatic cell count has shown similar improvements since it was included in the early 2000s (AHDB, 2020).

At farm-level, increases in milk yield per cow have driven improvements in production efficiency and there continues to be a trend towards intensification on a smaller number of specialised production units (van Arendonk and Liinamo, 2003; Capper *et al*., 2011). In 2003, it was estimated that intensive high input: high output dairy systems accounted for 85% of total milk production in the EU (van Arendonk and Liinamo, 2003). In the UK, while the total number of dairy cattle has decreased, the average dairy herd has increased in size from 75 to 148 between 1996 and 2018 (AHDB Dairy, 2020). This increase in herd size has necessitated a shift in management practices to allow stock people to manage a greater number of animals. One consequence of increased herd size is a reduction in time available for animal observation, which means that the detection of disease and oestrus is challenging.

Disease detection is performed intuitively by a good stock person as part of their general herd management. However, as average herd size and labour costs continue to increase, it is more difficult and time consuming for stock people to identify and treat sick cows in a timely manner (LeBlanc, 2010; Bicalho *et al*., 2007). These trends have fuelled an interest in automated systems of disease detection which can objectively monitor health in large herds and allow the identification of cows at high risk of disease (LeBlanc, 2010; Wathes *et al*., 2008). Lukas *et al*. (2009) suggested that even non-specific alerts to highlight individual animals requiring special attention would be beneficial. Such systems would enable stock people to implement appropriate and timely interventions in order to reduce the negative impact of disease on health, welfare and production. Examples of disease detection tools which are currently available include Herd Navigator™ for mastitis detection; StepMetrix™ for lameness detection and HiTag™ rumination collars (Rajkondawar *et al*., 2002; Chagunda *et al*., 2005; Schirmann *et al*., 2009).

Despite a prediction by Ingvartsen *et al*. (2003) that tools for the automatic detection of disease would become important on modern dairy farms, thus far uptake of reliable detection and monitoring aids, particularly for metabolic disorders, has been limited. Two main barriers to their further development persist. Firstly, uncertainty exists as to which indicators best reflect metabolic status and allow for the prediction of disease (Ingvartsen *et al*., 2003). de Vries *et al*. (2011) concluded that it was not clear which variables of routine herd data were associated with dairy cattle health and welfare indicators, and Rutten *et al* (2013) proposed that further research was need to identify appropriate on-farm indicators for metabolic diseases. In 2013, Moyes *et al*. described a new index of physiological imbalance which is based on plasma concentrations of non-esterified fatty acids, beta-hydroxybutyrate and glucose. Cows with higher physiological imbalance before calving were shown to have a greater disease risk in early lactation (Moyes *et al*., 2013). Further work is needed to develop automated on-farm disease detection systems; the generation of the physiological imbalance index relied on the collection of weekly blood samples, which is impractical (Moyes *et al*., 2016). Secondly, although technological advances mean that high frequency data for several production parameters is now available, a lack of suitable analysis means that this is not always converted into biologically meaningful information. Codrea *et al*. (2011) highlighted the need for mechanisms which allow the extraction of key information from large quantities of data. Opportunity also exists to exploit the wealth of data currently generated on farm, without the need for investment in specialised equipment or reliance on invasive techniques. Further, because most production disease occurs in the month after calving, the potential to record milk and blood measures in this short space of time is limited. Therefore, it may be of value to use pre-calving information i.e. data, which is recorded in the dry period, to identify cows at risk of early lactation disease several weeks before calving. Incidence of metabolic diseases in the early lactation period has been identified as one of the most serious welfare issues affecting dairy cows (von Keyserlingk *et al*., 2009).

1.2 Literature Review

1.2.1 The relationship between production and disease

There are complex and interdependent relationships between disease risk and milk production traits; many diseases have a symptomatic negative effect on milk yield which can mask the effect of a predisposing high milk yield pre-diagnosis (Fleisher *et al*., 1991). If it is true that selection for milk yield has increased disease incidence, it is reasonable to assume that the highest yielding cows in any given population will experience increased disease incidence compared to lower yielding cattle. Indeed, it was reported that cows with higher than average previous yields were at an increased risk of developing parturient and non-parturient paresis (Grohn *et al*., 1989). Similarly, Ostergaard and Grohn (1999) found high milk yield to be a risk factor for ketosis and enteritis, while Rajala-Schultz *et al*. (1999) reported that cows which contracted milk fever were among the higher yielding cohort. Fleischer *et al.* (2001) found a connection between yield in the current lactation and incidence of milk fever. High first test day milk yield has also been shown to increase the risk of ovarian cysts and lameness (Heuer *et al*., 1999). In contrast, Deluyker *et al*. (1991) reported that low milk yields in the first five days of lactation were associated with an increased incidence of early *postpartum* metritis; however low milk yield may be a symptom rather than a risk factor of metritis. The clearest relationship between disease and production exists between high milk yield and risk of mastitis. Bigras-Poulin *et al*. (1990) showed that an increased risk of mastitis was associated with high-producing cows. In their review, Ingvartsen *et al*. (2003) found that most epidemiological studies reported an increasing risk of mastitis with increasing previous lactation yield, and that the genetic correlations between milk yield and risk of mastitis were positive. Further, Windig *et al*. (2005) showed a clear relationship between milk yield, somatic cell count peaks and mastitis and concluded that a high milk yield increases the risk of mastitis.

The link between milk yield and lameness is also a subject of much debate in the literature which provides conflicting evidence of the impact that lameness has on milk yield. Green *et al*. (2002) reported that milk yield was reduced up to four months before diagnosis of lameness and continued up to five months after treatment, totalling an estimated reduction in yield of 360kg. Despite this prolonged reduction in milk yield, other studies have identified high milk yield as a risk factor for lameness. In particular, high milk yield in early lactation has been linked with an increased risk of lameness; in a retrospective cohort study using data from 2800 cows, cows subsequently diagnosed with clinical lameness had an average daily milk production of 33.3±0.31 kg in the first three weeks of lactation, compared to 30.1± 0.33kg per day for cows that did not develop lameness (Bicalho *et al*., 2008). It is not entirely clear as to why higher milk yield should cause lameness although various theories have arisen. Bicalho *et al*. (2008) *post*ulated that higher producing cows could be at an increased risk of laminitic type disorders because of subacute ruminal acidosis triggered by higher dry matter intakes than those seen in lower producing cows. More recently, the link between body condition score and lameness risk has been quantified and may explain some of the cause-effect relationship. Cows with a body condition score of less than 2 were demonstrated to have the highest risk of lameness due to decreased thickness of the digital fat cushion and therefore its reduced protective function (Randall *et al*., 2015).

One of the most critical side effects of increased production has been an increased level of metabolic stress for the individual dairy cow, particularly in early lactation (Oltenacu and Broom, 2010). Decades of genetic selection based solely on production traits have exploited the natural strategy of the dairy cow to prioritise energy supply for milk production in early lactation (Opsomer, 2015). In the immediate *postpartum* period, mammals prioritise milk production over maintenance of body functions, to the extent of mobilising body fat and protein reserves to ensure that the newborn's nutritional requirements are met (Opsomer, 2015). Selection for increased milk yield has increased energy requirements in the early lactation period, without proportionally increasing feed intake capacity (Opsomer, 2015). Although selection for higher milk yield increases feed intake somewhat, the correlated response in intake only accounts for half of the extra energy required for the increased milk yield - hence the gap between energy inputs and outputs widen, inducing greater mobilisation of body energy reserves (van Arendonk *et al*., 1991). Consequently, the duration and severity of negative energy balance in early lactation is increased (Veerkamp and Koenen, 1998; Ingvartsen *et al*., 2003). Dillon *et al*. 2004 showed that even when high genetic merit cows are supplemented with concentrates in early lactation, the extra energy provided is partitioned to milk production rather than to redress lipid mobilisation. This suggests that the widening energy gap in early lactation seen in high genetic merit cows can partially be attributed to genetically driven energy partitioning (Dillon *et al*., 2004).

The physiological conditions associated with energy imbalance predispose dairy cows to metabolic and infectious disease; it is well established that excessive mobilisation of body fat reserves and the consequent increase in plasma fatty acid concentration are significant risk factors for early lactation disease (Esposito *et al*., 2014; Sordillo *et al*., 2013). The growing imbalance between energy inputs and outputs in early lactation as a result of increased production, therefore, has a significant effect on the development of early lactation diseases including metabolic disorders, retained placenta, metritis, and mastitis and has implications for fertility and longevity. This is in addition to the "natural" physiological stress which a cow is subject to as she transitions from gestation to lactation. Associations between energy balance, cow health, milk production and reproduction will be examined more closely in Chapter 3.

1.2.2 The Dry and Transition Periods

A six to eight-week non-lactating interval prior to parturition ("dry period") is standard practice on dairy farms and is an essential component of dairy herd management. It is well documented that dry cow management has an important effect on lifetime health and productivity of the dairy cow (Kim and Suh, 2003). Arguably of even greater importance is the "transition period", first defined by Grummer in 1995, which extends from three weeks *prepartum* to three weeks *postpartum* and thus includes the final three weeks of the dry period. The transition period represents the most physiologically challenging period in the lactation-gestation cycle for the dairy cow- when it is required to give birth, initiate lactation and adapt to significant changes in diet, housing and social grouping (Drackley, 1999). Such is the importance of this period that failure to manage cows correctly through transition has serious consequences for both animal welfare and dairy farm profitability. Management of cattle during the non-lactating period is critical in determining future health and productivity (Dingwell *et al.,* 2001). The purpose of this section of the literature review is to gain an understanding of the biology of the transition period and current methods of managing dry and transition dairy cows.

1.2.2.1 Transition cow biology

Demand for glucose and metabolizable energy increases two to threefold as the dairy cow transitions from gestation to lactation (Drackley *et al*., 2001). This presents a major challenge for the cow – how can she consume sufficient energy to meet this increased demand at a time when dry matter intake is limited? Due to the lag in the increase in rumen capacity and dry matter intake, it is impossible for the cow to meet her energy demands from dietary supply (Bell, 1995). DMI of late gestation cows can be inhibited by up to 30% and is a result of various physical, behavioural, metabolic and hormonal changes around the time of calving (Dann *et al*., 1999; Contreras and Sordillo, 2011). The occurrence of health events and the effects of treatments administered to alleviate them can further limit DMI in the pre- and *postpartum* periods (Drackley, 1999). Therefore, cows in early lactation experience a negative energy balance (NEB) whereby it is necessary to mobilise body reserves to meet this increased energy demand. Hormonal changes facilitate the mobilisation of long chain fatty acids from adipose tissue to support lactation and circulate as non-esterified fatty acids (NEFA) in blood, the concentration of which is a useful indicator of the extent of body reserve mobilisation (Ingvartsen, 2006). These mobilised fatty acids make a significant contribution to the energy cost of milk production in early lactation (Friggens *et al*., 2004). Some controversy exists as to whether this period of body reserve mobilisation is actually a response to decreased feed intake or whether it is genetically driven and would occur even where nutrient supply was not limited (Friggens *et al*., 2004). There appears to be at least some component of genetically driven body reserve mobilisation as a result of a drive to prioritise milk production, pregnancy and accretion of body reserves at different stages of the lactation cycle, but mismanagement of late gestation and early lactation cows can exacerbate the period of negative energy balance (Friggens, 2003; Esposito *et al*., 2014). Furthermore, lipid mobilisation has in itself been suggested to be a cause of depressed feed intake due to its ability to promote satiety and decrease feeding behaviour (Allen *et al*., 2005).

The use of body tissue as an energy source, though essential in early lactation, is not problem-free. The liver has a limited capacity to metabolise fatty acids and as such, when its capacity has been reached, NEFA begins to accumulate as triglycerides in the liver. This further impairs liver function and initiates the production of ketone bodies such as acetoacetate and beta-hydroxybutyrate (BHB), indicative of ketosis (Goff and Horst, 1997; Suthar *et al*., 2014). This impairment of the liver also inhibits glucose production which means that a state of hypoglycaemia ensues in which dysregulation of the normal insulin response occurs in order to allow any glucose that is manufactured to be prioritised for milk production in the mammary gland (Abuelo *et al*., 2015).

Concurrent with, and related to, the energy challenge in early lactation, is a well-recognised period of immune suppression where the cow's ability to combat pathogens is compromised. Franklin *et al*. (1991) found that *in vitro* levels of acetate associated with ketosis suppressed lymphocyte function to an extent which was likely to influence the immune response to ketosis *in vivo*. Furthermore, in their review, Goff and Horst (1997) identified increases in oestrogen and glucocorticoids in early lactation as likely causes of *postpartum* immune suppression. Of particular interest is the work of Lactera *et al*. (2005) who found that cows that experienced more mobilisation of body lipid reserves (NEFA levels exceeding 600 µmol/L) had significantly impaired lymphocyte function compared to cows with NEFA levels less than 600 µmol/L; suggesting a direct effect of the mobilisation of body lipid reserves on immune function. In addition to impairing lymphocyte function, the mobilisation of body lipid reserves has been shown to alter metabolic pathways such that cows are more likely to initiate excessive inflammatory responses which are pathophysiological in the development of transition cow diseases such as metritis and mastitis (Contreras and Sordillo, 2011). Trevisi *et al*. (2012) found that incidence of disease in the 30 days pre- and 30 days *post*-calving in high yielding cows was correlated with signs of an accentuated inflammatory response, in particular an increase in Interleukin-6. With regard to specific diseases, the level of circulating inflammatory biomarkers has been shown to be 2.3 times greater in ketotic cows compared to healthy cows (Abuajamieh *et al*., 2016). A further factor in the interrelationship between energy status and immunity is that an activated immune system, such as one that is mounting an inflammatory response, has been shown to have a high glucose demand (>1kg within 12 hours) (Kvidera *et al*., 2017).

When NEFA is used as an energy substrate, production of oxidants (reactive oxygen species (ROS)) is enhanced thus inducing a state of oxidative stress (Abuelo *et al*., 2015). Oxidative stress has two major impacts on the cow; firstly, it causes additional mobilisation of body lipid reserves which causes a vicious cycle of mobilisation and the production of ROS (Sordillo and Raphael, 2013; Abuelo *et al*, 2015). Secondly, increased levels of ROS have been identified as a risk factor for the development of diseases associated with early lactation. In an observational study of 24 Holstein cows throughout the transition period, it was found that the degree of oxidative stress experienced by the cow was related to energy status; cows with a higher body condition score and greater losses of body condition in early lactation were more sensitive to oxidative stress (Bernabucci *et al*., 2005).

A review of the current literature related to transition cow biology highlights the complexity of the physical, metabolic and hormonal changes that occur in the late gestation and early lactation period. The interplay between mobilisation of body reserves, immune and inflammation dysregulation and oxidative stress has been termed the “metabolic stress triad” by Abuelo *et al*., 2019, which helpfully synthesises current understanding on transition cow biology. Future fundamental research in lipid science and applied research to develop a standardised method of assessing oxidative status in dairy cattle would allow the development of management strategies and nutritional approaches to improve transition cow health (Contreras and Sordillo, 2011; Abuelo *et al*., 2019).

1.2.2.2 Management of non-lactating cows

The primary purpose of the dry period is to ensure optimal milk production efficiency in the next lactation by allowing mammary gland involution, and to cure and prevent intramammary infections whilst in a non-lactating state (Pezeshki *et al*., 2008; Henderson *et al*., 2016). This process has been demonstrated to improve milk yields – conversely, omission of the dry period has consistently been associated with losses in milk production of up to 24% when compared to cows with an eight-week dry period (Heeren *et al*., 2014). Secondary to managing udder health, because the energy demands for milk production are removed, dry cows are able to modulate their body energy reserves in preparation for calving and subsequent lactation (Friggens *et al*., 2004).

Much research has been undertaken to establish best dry cow management practices, with the majority focusing on dry-off procedure, length of the dry period length and nutritional management strategies for use in the dry period.

(i) Dry-Off Procedure

Drying off refers to the process used to artificially terminate lactation and thus begin the dry period. Typically, modern dairy production dry-off protocols involve dietary and milking routine changes designed to reduce milk synthesis (Franchi *et al.*, 2019). The two main strategies for drying-off are either an abrupt cessation of milking or a gradual reduction in milking frequency with the intention to reduce milk production (e.g. reducing to once a day milking for a week before dry-off). Controversy exists as to which method offers the best outcomes relative to udder health and cow welfare. The principal risk associated with poor management of dry off is the establishment of intramammary infections (Dingwell *et al.*, 2001). It has been estimated that 52% of clinical cases of environmental mastitis in early lactation originate from infections established in the dry period (Bradley and Green, 2000).

The abrupt cessation of milk removal to initiate the non-lactating dry period is a routine management practice in dairy herds (Zobel *et al.*, 2013). In a recent study of German dairy farmers, 73.0% performed abrupt dry off (Bertulat *et al.*, 2015). Abrupt dry-off has historically been recommended by veterinary surgeons and as such, its practice is widespread (Blowey and Edmondson, 2010; Bertulat *et al.*, 2013). It continues to be recommended by a variety of dairy related businesses, including manufacturers of milking equipment (e.g. Lely). A survey of 116 UK dairy farms conducted by Fujiwara *et al.* (2018) reported that 83% practiced abrupt dry-off. However, the increased lactation yield of modern dairy cattle means that it is increasingly necessary to dry off cows whilst they are still producing significant quantities of milk – often in the region of 25-30kg/day (Stefanon *et al.*, 2002). Research conducted over the last decade suggests that this practice can cause discomfort and distress to cows (Zobel *et al.*, 2013). Bertulat *et al.* (2013) reported that abrupt dry-off had a negligible effect on cows yielding less than 15 kg/day, but cows with a yield greater than 20 kg/day suffered high intramammary pressure and produced an increased level of the stress hormone, glucocorticoid. Odensten *et al.* (2007) found abrupt dry-off to be associated with increased intramammary pressure which in turn causes milk leakage and delays teat canal closure, thus increasing risk of infection. Further, Silanikove *et al*. (2013) studied the differences between high producing cows that had spontaneously reduced milk towards the end of their lactation to less than 14 litres, and high producing cows that were abruptly dried off whilst producing yields of 25-35 litres. Their data indicated that the abrupt mammary gland involution induced in cows dried-off abruptly provoked “signs of distress”, including engorgement of the udder and the presence of a higher than normal number of neutrophils in their milk.

Behavioural changes indicative of compromised welfare (e.g. reduced lying time) have also been reported following abrupt dry off (Chapinal *et al*., 2014). Zobel *et al*. (2013) recommended that gradual cessation of milking should be considered as a method of dry-off, especially for high-producing cows, as a gradual reduction in milking reduced milk leakage in their study. More recently, Gott *et al*. (2016) conducted a survey of 428 cows across 8 herds in the United States to determine the effect of dry-off procedure on milk yield and SCC in the following lactation. In their study, the method of cessation was not significantly associated with either milk yield or SCC in the first 120 days of the subsequent lactation, although its effect varied from herd to herd. In addition to herd effects, parity effects were also reported; abrupt drying-off of cows ending their first lactation was associated with an increased risk of mastitis at calving, whereas gradual cessation of milking increased mastitis risk in *multiparous* cows (Gott *et al*., 2016).

High milk yield at dry-off is itself a risk factor for future intramammary infection and high somatic cell count, and therefore the benefit that gradual cessation of milking is reported to have on measures of udder health when compared to abrupt dry-off, may be due to the reduction in milk yield achieved prior to dry-off (Rajala-Schultz *et al*., 2005). It has previously been reported that cows which have not had a significant reduction in milk yield before dry off have higher levels of intramammary infection compared with cows whose daily yield had reduced in the period before dry off, although the optimal level of production at dry off is not clear (Dingwell *et al*., 2001). Cows with a milk yield of greater than 20 kg/day in the 30 days before dry-off have an odds ratio of 1.29 for developing a high somatic cell count (>199,000 cells/ml) in the 30 days after calving (Green *et al*., 2008). Increased propensity for milk leakage and a reduction in natural defence mechanisms against infection amongst high yielding cows have been identified as possible reasons for such cows to be at greater risk of mastitis. In a study by Odensten *et al*. (2007), the proportion of cows with intramammary infections in early lactation was significantly lower amongst cows producing 5.0 - 11.4 kg/milk per day in the last week of lactation compared to cows producing more than 11.4 kg/day. The formation of the keratin plug, a key natural defence mechanism against new infections, has also been found to be compromised by high milk yield at dry-off (Dingwell *et al*., 2004; Rajala-Schultz *et al*., 2005). Pinedo *et al*. (2012) also found that previous cases of clinical mastitis and the presence of gram-negative bacteria in milk at the time of dry off significantly increased the risk of clinical mastitis within 30 days of calving.

A reduction in milking frequency and feed restriction are 2 management practices which can be used in combination or alone to achieve the necessary drop in milk yield prior to dry-off. Zobel *et al*. (2013) demonstrated that a gradual reduction in milking frequency reduced *post*-dry off milk leakage and thereby had a protective effect against bacterial infection. Gradual reduction in milking frequency, a reduction in milk yield and the use of intramammary antibiotics or teat sealants can reduce the risk of intramammary infection and thereby reduce the risk of clinical mastitis in the next lactation (Dingwell *et al*., 2001). Intermittent milking prior to dry-off is particularly important as it results in more rapid involution of mammary tissue and increases the production of lactoferrin, a natural bactericidal found in milk (Newman *et al*., 2010). Severe feed restriction immediately prior to dry-off reduces milk yield and can be achieved by either limiting the quantity fed or reducing the energy density of the diet. Restricting intake by limiting the quantity of feed offered presents a welfare challenge, as well as having potential to create metabolic problems (Valizaheh *et al*., 2008). Tucker *et al*. (2007) reported that cows fed 8kg DM of pasture and silage per day in the last 2 weeks of lactation spent less time eating, more time lying and had increased vocalisations compared to cows with an intake of 16 kg DM per day. Previous work had shown that late lactation cows offered a straw-only diet had increased signs of metabolic stress, including elevated blood NEFA concentrations and a reduction in rumen pH (Odensten *et al*., 2005). More recently, Dancy *et al*. (2019) conducted a study in which cows were assigned to be fed either a low (6.19 MCal/kg) or high nutrient (6.48 MCal/kg) density for the 5 days before dry-off. Cows fed the low nutrient density diet consumed an average of 2.2kg DM less per day and sorted the diet to a greater extent compared to cows fed the high nutrient density diet without any significant effect on cow physiology.

Several new approaches to dry-off procedures are now being reported in the literature. One method described in the literature is the administration of cabergoline. First described by Bach *et al*. (2015), administration of this ergot derivative was found to block prolactin secretion thereby causing a reduction in udder engorgement, milk leakage and an increase in lying time during the first two days after dry-off. These results were supported by the findings of Boutinaud *et al*. (2016) who reported that a single injection of cabergoline at the time of dry-off accelerated mammary involution and increased the presence of mammary gland defence mechanisms earlier than seen in control animals. Udder pressure, milk leakage and signs of udder pain were also significantly reduced following administration of cabergoline in a study conducted by Bertulat *et al*. (2017) using 234 cows. On the first day after dry-off, udder pressure in placebo and cabergoline treated cows increased by 115% and 42.3% respectively and 21% of placebo cows showed milk leakage compared to 11.3% of treated cows (Bertulat *et al*., 2017). In a study of 900 cows across several European countries, Hop *et al*. (2019) reported that the risk of developing new intramammary infections in the dry period and *post* calving was reduced by 21% in cows treated with cabergoline compared to those that received a placebo treatment. A number of questions regarding the long-term effects of cabergoline on udder health remain to be addressed. However, in the light of the above research, it appears conceivable that in the future, cabergoline could be one of the tools in the dairy producer's "dry-off toolkit".

The use of automatic milking systems (AMS) presents another opportunity with regards to refining end of lactation management and drying-off. Recent work by Martin *et al*. (2020) outlines a method which allows an effective stepwise reduction in milk yield without compromising udder health. Software was developed that allowed the degree of udder emptying to be gradually decreased by early removal of the milking clusters for 10 days before dry-off. The yield of cows subject to the experimental program was 35.3% less than control cows immediately prior to dry-off, with no negative short-term effects on somatic cell count or yield and udder health in the following lactation (Martin *et al*., 2020).

In addition to the challenge of high yields at dry-off, a requirement to reduce the routine administration of intramammary antibiotics at the time of dry-off is presenting a further challenge to dairy producers. For over five decades, following work by Smith *et al*. (1966), the use of intramammary antibiotics at dry-off was recommended as part of a five-part plan designed to reduce the incidence of mastitis. Studies which demonstrate the efficacy of antibiotics in preventing and curing intramammary infections in the dry period are well documented (e.g. Browning *et al*., 1993; Sol *et al*., 1994, Williamson *et al*.,1995, Hassan *et al*., 1999; Berry and Hillerton, 2002). However, the practice of blanket antibiotic treatment has come under increasing scrutiny and in some parts of Europe has been outlawed (Scherpenzeel *et al*., 2016). Selective dry cow therapy (SDCT) has come to the fore as a dry cow management strategy whereby only cows with evidence of chronic or current intramammary infection are treated with antibiotics at dry-off. Cameron *et al*. (2014) reported that SDCT achieved the same level of success with respect to treatment and prevention of intramammary infection over the dry period and mastitis risk in the first 120 days of lactation as did blanket treatment.

(ii) Dry period length

Since the beginning of the 20th century, the recommended length of the dry period has been six to eight weeks (Arnold *et al*., 1936), although this has and continues to be variously challenged. As in the case of dry-off procedure, increasing milk production has further necessitated a re-examination of the optimum length of the dry period (Grummer and Rastani, 2004; Pezeshki *et al*., 2008).

Many existing studies in the broader literature point to the fact that shortened dry periods reduce milk yield in subsequent lactations. For example, a study by Atashi *et al*. (2013) based on data from over 40,000 cows concluded that shorter dry periods were not beneficial to dairy production; cows with a dry period of 51 – 60 days produced more milk in the subsequent lactation than cows with a dry period of less than 50 days. More recently, O’Hara *et al*. (2020) reported that in their observational study, cows with a dry period of less than 39 days or greater than 80 days had the lowest milk yields. An observational study of over 70,000 Jersey cows concluded that dry periods of 45-70 days maximised yields across adjacent lactations and recommended that dry periods more than 70 days or less than 45 days are avoided

(Kuhn *et al*., 2007). However, these studies all used observational data meaning that the length of the dry period was not necessarily planned for but rather may have arisen from unexpectedly long or short gestation lengths, abortions, or errors in the recording of fertility events. Thus, these cows were not managed with regard to having an abnormally short or long dry period.

Results from studies where cows were assigned to dry periods of particular lengths are inconsistent. Gulay *et al*. (2003) reported no significant differences in mean daily milk yields in the first 21 weeks of lactation between cows that had a 30-day dry period and cows that had a 60-day dry period. Similar results were obtained by Pezeshki *et al*. (2007) who reported that, for *multiparous* cows, no difference in milk yield was detected between cows assigned to a dry period of 56 days compared to those assigned to a 35-day dry period. Interestingly, they did report some parity effects – first lactation heifers with a 35-day dry period produced significantly less milk than those with a 56-day dry period. Thus, their conclusion was that shortened dry period may be beneficial for *multiparous* or overfat cows but is not recommended for first lactation animals (Pezeshki *et al*., 2007). On the other hand, Steeneveld *et al*. (2013) used data from five Dutch farms who had planned for different lengths of dry period and found that milk yield of cows with a dry period length of less than 20 days was 5.7 – 13 kg/day less than cows with a dry period of longer than 35 days. The extremely short dry period length employed in this study may have elicited the significant milk yield loss which was not seen in previous studies due to less extreme dry period lengths. Comparable results were obtained by van Knegsel *et al*. (2014) who reported that average milk yield until week 14 of lactation was 10.6kg/day and 4.6kg/day lower for cows with 0- and 30-day dry periods respectively, when compared with yields of cows with a “normal” 60 day dry period.

As outlined, the effect of dry period length on milk production is complex and variable and is not yet fully understood. However, a further driver for re-examining optimal dry period length is its effect on early lactation health (Grummer and Rastani, 2004). Research has provided evidence that shortening or even omitting the dry period has a positive effect on energy balance in early lactation and in some cases, reduces disease incidences and lessens the problems associated with the transition period (Grummer *et al*. 2010). In 2008, Watters *et al*. assigned cows to either a 55- or 34-

day dry period and found that those assigned to the shorter dry period had significantly lower NEFA concentrations *postpartum*. However, there was no difference in the incidences of ketosis, retained placenta, displaced abomasum or metritis. Grummer *et al*., (2010) reported similar effects. They found that cows with no dry period did not experience any negative energy balance in early lactation, as a reflection of lower milk production and greater feed intake in this period (Grummer *et al*., 2010). Similarly, *postpartum* negative energy balance was reported to be less severe in cows with no dry period compared to those with 30- or 60-day dry periods (van Knegsel *et al*., 2014). With reference to health events, cows with a dry period of 30 -39 days had an odds ratio of 1.9 for retained placenta relative to cows that had a dry period of 60-69 days, and cows with a dry period of between 40 – 59 days had the lowest risk of culling in the following lactation (O'Hara *et al*., 2020). Physiological indicators of stress have also been reported to be influenced by dry period length. Measures of oxidative stress including levels of ceruloplasmin and cholesterol were increased in cows with no dry period compared to those with a 30–60-day dry period. However, no difference in health problems were reported between groups (Mayasari *et al*., 2019).

In the light of reported associations between energy balance and *postpartum* resumption of ovarian cyclicity (e.g. Lucy *et al*., 1991), it is possible that shortening the dry period and thereby reducing negative energy balance would also affect fertility. However, conflicting results as to the effect of dry period length on fertility are reported in the extant literature. A beneficial effect of shortening the dry period was reported by Watters *et al*. (2009); services per conception were 8% lower in *multiparous* cows with a dry period of 34 days compared to *multiparous* cows with a 56-day dry period. This was reinforced by findings which showed that omitting the dry period increased the normal resumption of ovarian activity compared with a conventional 60-day dry period (Chen *et al*., 2015). In contrast, results from Chen *et al*. (2017) suggest that dry period length was not associated with uterine health status in early lactation and thus had no effect on fertility. O'Hara *et al*. (2020) reported no effects of dry period length on conception rate at 1st service or on calving to first insemination interval but did see negative effects on pregnancy rate in cows with a dry period greater than 70 days.

Even though there are some positive effects of shortening or omitting the dry period on energy balance in early lactation, the significant negative effect it has on subsequent milk yield cannot be ignored. The conflicting findings in this area signal the need for additional studies to more fully understand the effect of shortening or omitting the dry period on long-term productivity, health, and fertility. Increasing uptake of technology and increasing herd size may in the future present opportunities to tailor dry period length to individual cows according to parity, calving interval, or milk yield (Grummer and Rastani, 2004)

(iii) Nutritional management of dry and transition cows

(a) Energy level and fat supplementation

When cows leave the milking herd and begin the non-lactating stage of the production cycle, they experience a sudden and major dietary change. The rumen environment must adapt to the change from an energy dense lactation diet to a diet which meets basic maintenance requirements, before preparation begins during the transition period to adjust back to the lactating ration (Dingwell *et al.*, 2001). Concerns have been raised that such a major shift in nutrient supply at dry-off may lead to metabolic disorders in the transition period and ensuing lactation, especially among high-yielding cows (Odensten *et al.*, 2007). This may be explained by the resultant changes in body energy reserves following drastic dietary change; *prepartum* negative energy balance is known to be a significant risk factor for *postpartum* displaced abomasum (Cameron *et al.*, 1998). Critically, mobilisation of body energy reserves in early lactation is directly influenced by the pattern of accretion and mobilisation of reserves experienced in the dry period (Garnsworthy and Topp, 1982). Traditionally, it was recommended that the energy and nutrient density of dry cow rations be maintained at a high level throughout the dry period. Termed, "steaming up", this strategy sought to accustom the cow to the “high grain” diet she would be fed after calving and to build up her energy reserves (Greenhalgh and Gardner, 1957). However, more recent research has shown that such an approach has significant effects on body energy reserves in the immediate pre and *postpartum* period. When high energy and controlled energy dry period diets are compared, high energy rations have been seen to induce greater lipid mobilisation in the *postpartum* period (Mann *et al.*, 2015). Cows consistently fed a diet which exceeds their energy requirements throughout the dry period and those fed a restricted energy diet in the far-off dry period (from dry off until 28 days before predicted calving date) and a moderate energy diet in the close-up dry period (within 28 days of predicted calving date) have been shown to have an increased incidence of ketosis in early lactation (Mann *et al*., 2015). *Prepartum* diets which have an energy density greater than 6.9 MJ of net energy per kilogram of dry matter have been identified as significant risk factors for *postpartum* displaced abomasum and ketosis (Cameron *et al*., 1998; McArt *et al*., 2013). Importantly, controlled energy diets in the dry period have proved to be successful in minimising the degree of negative energy balance *postpartum* as well as decreasing the incidence of ketosis. This research led to updated recommendations for dry period nutrition which focus on ensuring cows are not excessively fat at calving and limiting energy intake in the 2 weeks before calving (Roche *et al*., 2013).

However, in contrast to most of the work in this subject area, Salin *et al*. (2018) reported no beneficial effects of restricting *prepartal* energy intake on body weight and condition losses in early lactation. In their study, high yielding Ayrshire cows were assigned to one of 2 diets which were formulated to provide 108% or 141% of daily energy requirement and were composed of grass silage or a TMR, supplemented with a pelleted concentrate. Based on their results, the authors *post*ulated that cows on grass silage based diets before and after calving are not prone to large changes in either body condition or body weight after calving, and infer that the composition of dry cow diets may affect patterns of body weight change to at least the same degree as total energy intake (Salin *et al*., 2018).

Supplementation of dry cow diets with fatty acids has also been proposed as a strategy to improve health and productivity outcomes in early lactation (Andersen *et al*., 2008). Due to the limited capacity of the bovine liver to process fatty acids, when body reserves are mobilised in early lactation, liver function is compromised and diseases such as fatty liver and ketosis can occur. However, in their review of dry cow feeding strategies, Friggens *et al*. (2004) suggested it is likely that based on the evidence at the time, the capacity of the liver to process fatty acids is improved by exposure to increased concentrations of fatty acids in the blood. Thus, in theory feeding a high fat diet in the dry period would increase circulating levels of fatty acids and therefore prepare the cow to cope with the inevitable increased fatty acids after calving (Friggens *et al*., 2004). In an experiment using 27 cows, Andersen *et al*.

(2008) found that whilst being fed either a high saturated fat or high unsaturated fat diet in the dry period, levels of circulating fatty acids were higher than those seen in cows fed a low fat diet. Cows fed the high saturated fat diet had the lowest level of circulating fatty acids in early lactation, and thus the authors concluded that supplementing dry cows with a fatty acid source is a valid approach to preparing cows for early lactation (Andersen *et al*., 2008). Conflict exists in more recent literature as to the effects of fat supplementation in the dry period. In a study using a similar number of cows to Andersen *et al*. (2008), Karimian *et al*. (2015) reported that supplementation of dry cow diets with calcium salts of fatty acids (1.6% of DM) produced no benefits; metabolic indicators of stress in early lactation and production were not significantly different compared to cows that received no fat supplementation. However, more recently supplementation of a low starch close-up dry diet with 20g/kg DM of a fat supplement high in stearic acid was shown to increase *postpartum* dry matter intake relative to body weight (Daneshvar *et al*., 2020). Further research is needed to consolidate understanding of the effects of fat supplementation in the dry period on measures of health and productivity in early lactation, and to more fully understand the differences between fatty acid types and to tease out the apparent interactions between fat supplementation and diet type (e.g. high and low starch).

(b) Protein level and amino acid supplementation

NRC recommendations in 2001 estimate that 820 grams of protein are required by a cow approaching calving, with an expected calf birth weight of 42kg; in practice, where dry matter intake is not limited, this can be achieved by feeding a diet of between 11 -13% crude protein (NRC, 2001; Husnain and Santos, 2019). However, recent research suggests that transition cows may benefit from balancing amino acid supply (Schwab and Broderick, 2017). A growing body of work points to the effects that both the overall level and type of protein in dry cow diets have on performance and health in early lactation, with reference to amino acid supply. The literature offers contradictory findings about the benefits of supplementation with specific amino acids.

Methionine supplemented dry cows have been reported to have improved DMI in early lactation (an improvement of 2.1kg per day) and tended to have a lower incidence of ketosis coupled with a higher concentration of blood neutrophil phagocytosis, suggesting improved immune function (Osorio *et al*., 2013). The extent to which methionine supplementation affects DMI was very similar in a study by Zhou *et al.* (2016) who found that it increased DMI by 1.1kg per day, however this was in the *prepartal* period. Additionally, they demonstrated that cows supplemented with methionine tended to have a lower incidence of ketosis and retained placenta suggesting a better transition between gestation and lactation (Zhou *et al.*, 2016). In 2017, Batistel *et al.* found that methionine supplementation improved DMI by 1.7kg/day in early lactation and supplemented cows "tended to have better liver function" – circulating fatty acids were reduced compared to cows that were not supplemented (p = 0.08).

Supplementation with choline in the dry and transition periods has also been shown to improve liver function by metabolising mobilised adipose fat into a form which can be transported and used around the body as a fuel source and thus prevent its accumulation in the liver (Sun *et al.*, 2016). In 2007, Cooke *et al.* showed that supplementation with rumen protected choline decreased fat (triacylglycerol) deposition in the liver and concluded that such supplementation can prevent and possibly alleviated fatty liver syndrome which is induced by feed restriction. In a study by Sun *et al.* (2016) supplementation in the dry period significantly reduced plasma NEFA and BHB concentrations in early lactation by 0.3mmol/L and 0.1mmol/L, respectively. Conversely, Hartwell *et al.* (2000) and Zahra *et al.* (2006) did not report any positive effects on liver metabolism of *prepartum* choline supplementation – although in the case of Hartwell study, the degree of rumen protection of the choline has been questioned. A recent meta-analysis using data from 21 experiments concluded that choline supplementation increased *pre* and *postpartum* dry matter intake and milk yield and 'tended' to reduce risk of retained placenta and metritis but had no effect on the incidence of metritis, milk fever, left displaced abomasum, ketosis or triacylglycerol levels in the liver (Arshad *et al.*, 2020). Different results in these studies are likely to be because of various different approaches to feeding the supplement i.e. top dressing or mixed in a TMR, different lengths of feeding and different basal diets.

Regarding overall protein content, most of the research has sought to quantify production effects rather than the effect that dry cow protein nutrition has on *postpartum* health. An extensive meta-analysis of the effects of *prepartum* protein on subsequent cow performance was recently conducted by Husnain and Santos (2019). Increased dietary protein in the dry period increased feed intake both before and after calving and *prepartum* body condition score in heifers however, this was not seen in *multiparous* cows. They concluded that feeding heifers a pre-calving diet of 14 to 15% crude protein improves lactation performance, whereas no additional benefits would be achieved by increasing crude protein supply to cows, except for an increased milk protein content in cows yielding greater than 36kg of milk per day (Husnain and Santos, 2019). In what appears to be the first study to examine the interactions between dietary protein content in the pre and *postpartum* periods, cows fed a higher crude protein (15% vs. 12%) in the *prepartum* period tended to have an increased DMI in the dry period and required less crude protein to maintain milk yield in early lactation, than those fed the lower level in the dry period (Amirabadi Farahani *et al.*, 2019). In relation to health and metabolism in early lactation, the study also showed that increasing the crude protein of the dry period diet from 12 to 15% decreased serum BHB concentration in early lactation (Amirabadi Farahani *et al.*, 2019). Although not stated in the study, this suggest that cows fed a high level of crude protein in the dry period were "metabolically healthier" and less likely to suffer from ketosis in early lactation, compared to those fed a low protein dry period diet.

(c) Mineral nutrition

Mineral nutrition in the dry period is also of critical importance in determining health outcomes in early lactation. Of particular importance is its role in preventing parturient paresis or "milk fever" (Friggens *et al.*, 2004). Although milk fever does not result from a nutritional deficiency of calcium, prevention strategies centre on nutritional management. Management to decrease the risk of milk fever is critically important as it is widely understood that milk fever is a risk factor for other diseases including ketosis, left displaced abomasum, metritis and retained placenta and its incidence is reportedly around 5 – 10% (Cardoso *et al.*, 2020). Traditionally, milk fever risk has been managed by limiting calcium intake in the *prepartum* period to induce calcium release from body reserves. In practice this has become increasingly difficult due to the typical high calcium content of forages which means that calcium requirements in the late dry period are often exceeded by the forage component of the di*et al*one (Thilsing-Hansen and Jorgensen, 2001). In their study comparing different levels of calcium, Goff and Koszewski (2018) found that a pre-calving diet with 0.46% calcium was not low enough to stimulate calcium release before calving to prevent hypocalcaemia.

In the United States, the most common milk fever prevention strategy is the Dietary Cation-Anion Balance (DCAB) system which balances dietary minerals to create slightly acidic conditions in the blood that promotes calcium mobilisation from bone reserves (Pehrson *et al*., 1998). In their meta-analysis, which include 22 published studies, Charbonneau *et al*. (2006) found that reducing DCAB from +300 to 0 mEq/kg reduced the risk of clinical milk fever from 16.4% to 3.2%, by successfully inducing metabolic acidosis. Interestingly, very few of the studies included reported any effect of DCAB on DMI, however the meta-analysis did find a significant negative effect which could be caused by reduced palatability of the low DCAB diets as a result of the anionic salts used to achieve this. Most studies conducted in this field agree as to the effectiveness of reducing DCAB to prevent milk fever; one of the most recent studies by Glosson *et al*. (2020) confirmed previous findings that an acidogenic *prepartum* died improved calcium status and health in early lactation. Wu *et al*. (2008) fed 4 diets of -50, -150, +50 and +150 mEq/kg DM in a random block design to 40 Holstein cows, and although no cows in the experiment developed clinical milk fever, cows fed the negative DCAB diets had significantly greater blood calcium levels *postpartum* and had reduced incidence of retained placenta compared to the cows fed the +150 mEq/kg DM diet.

Another approach to managing mineral nutrition with a view to minimising the incidence of milk fever is the inclusion of zeolite feed supplements in pre-calving diets. They work by adsorbing dietary calcium, making it unavailable for absorption by the cow and thus initiating the upregulation of calcium metabolism as necessary as calving approaches (Crookenden *et al*., 2019). In their small scale study using 17 Jersey cows, Thilsing-Hansen and Jorgensen (2001) found that no cows fed a synthetic zeolite developed clinical or subclinical milk fever; however, 3 out of 8 of the cows not fed the zeolite developed clinical milk fever with 6 out of 8 showing signs of subclinical disease. A larger scale study using 55 Holstein cows demonstrated improved blood calcium levels in the period immediately after calving and a decreased incidence of subclinical milk fever (Kerwin *et al*., 2019). However, some negative effects were reported including depressed intakes and rumination in the *prepartum* period in cows fed the supplement.

(d) Conclusions on Dry Cow Feeding

Dry cow nutrition continues to be an extensively researched topic, particularly with reference to improving health and productivity outcomes in early lactation. However, no single nutritional strategy has been repeatedly shown to provide consistent outcomes for transition cows (van Saun and Sniffen, 2014). Despite this, there are some general principles which can be identified in the literature. Regarding energy supply, the consensus of the most recent research appears to be that controlled energy diets offer the best outcomes in early lactation, so long as cows do not experience hunger. Supplementing dry cow diets with fat has shown to effectively prepare the liver for lactation, however it is essential that their usage does not mean that dietary energy is over-supplied and more research is necessary to fully understand the interaction between fat supplementation and diet type. Improving the quality of protein supply in the dry cow ration has a clear positive effect on productivity in early lactation, although its effects on health are less well understood. Accurately formulating the mineral content of dry period diets is essential to minimise the risk of milk fever – different approaches to control calcium metabolism are available and are valid for use in different feeding situations.

1.2.3 Monitoring health status and disease risk

In human medicine, the importance of early disease detection in successful therapy is well recognised; in general, the earlier the disease is diagnosed, the more likely it is to be successfully cured or controlled, and the less impact it is likely to have on the patient's life (Lee and Wong, 2009). Similarly, in veterinary medicine, prediction or early detection of disease is an important goal as it allows targeted and swift interventions aimed at reducing the negative effects of disease on cow health and welfare and minimising economic losses (LeBlanc., 2010). Milner *et al*. (1997) showed that rapid detection of mastitis in goats resulted in high bacteriological cure rates, and Zimmerman (2001) demonstrated that prompt identification of lameness in cattle prevented the development of chronic conditions. Therefore, the goal of any health monitoring system should be to monitor the success of current management in order to identify any problems or deviations from the planned management program, and to identify cows at high risk for disease in order that clinical disease may be mitigated (LeBlanc, 2006).

Advances in technology now mean that it is possible to routinely record and store production data for individual cows (Codrea *et al.*, 2011). This has increased the opportunity for real-time monitoring of health status; potential exists to identify aspects of recordable traits which are reflective of health status and may be used to calculate individual cow disease risk. The use of data which is already available on farm has the advantage of being cheap to obtain and does not require specialist equipment. Further, the identification and use of indicators of health status which do not require invasive techniques (i.e. where equipment does not need to be attached to or inserted into the animal) have clear welfare and safety benefits (Mottram, 1997). This section of the review will outline key sources of potential disease indicators from data, based on their potential ability to distinguish between healthy and sick cow and give a brief overview of current health monitoring systems.

1.2.3.1 Sources of health status indicators

(i) Milk yield and quality

Milk is an obvious source of information about the health of the cow both through yield and analysis of its composition (Mottram, 1997). Additionally, milk conductivity and milk yield acceleration (the rate of change in yield) have also shown potential as indicators of disease. As previously outlined, the relationship between milk yield and disease is complicated. In general, health disorders lead to a decrease in milk yield, the magnitude of which is disease dependent (Barielle *et al.*, 2003). In terms of the effect of disease, milk yield of ketotic cows has been shown to decline as early as 4 weeks pre-diagnosis, with milk yield losses ranging between 3-5 kilograms per day, depending on parity (Rajala-Schultz *et al.*, 1999). Additionally, first test-day milk yield was shown to be 7.1 kg lower after diagnosis of displaced abomasum (Heuer *et al.*, 1999). Fourichon *et al.*, (1999) found that for DA, average milk yield losses ranged from 400 to 800 kg. Retained placenta and early metritis (within 28 days of calving) were both found to significantly affect milk yield, as measured by monthly test-day yields (Rajala and Grohn, 1998). For cows with mastitis, daily milk yield losses during the 2 weeks after diagnosis are reported to vary from 1.0 – 2.5 kg with total lactation losses of 110 – 552 kg, dependent on parity and the time of mastitis occurrence

(Rajala-Schultz *et al*., 1999). Barielle *et al* (2003) found that, on the day of diagnosis, mastitis, ketosis and milk fever were associated with a significant decrease in milk production of 4.1 – 25.7 kg. Hostens *et al*. (2012) showed that cows diagnosed with one metabolic disease did not have significantly lower yield than healthy cows; level of production was only lowered in cows that were affected by multiple metabolic diseases. In addition, cows with multiple metabolic diseases were shown to have a slower rise to peak yield in early lactation, which was compensated for by greater yield consistency (Hostens *et al*., 2012). Despite being of some relevance in understanding the underlying physiology, quantifying the effects of disease on milk yield does not necessarily provide the opportunity to identify cows at future risk of disease - when milk yield has already begun to decline the disease has developed. Real-time data which relates more to future disease risk such as milk yield acceleration may be of greater value in identifying at risk cows. Milk yield acceleration (MYAcc) has been suggested for use as an index of physiological stress, especially in early lactation when MYAcc and disease incidence are both at their highest level of the production cycle. The trait was first suggested as an indicator of health status following the demonstration of an association between MYAcc and conception rate (Domecq *et al*., 1997). High MYAcc has been linked to an increased risk of an imbalance in fat and carbohydrate metabolism and thus increases the risk of ketosis (Nielsen *et al*., 2005). Moreover, Chiumia *et al.* (2013) found that high MYAcc after calving exposed cows to a higher risk of involuntary culling due to udder health problems.

As alluded to in section 1.2.1, an apparent contradiction exists; high yielding cows are more prone to metabolic disease, yet as seen here, metabolic disease will reduce milk yield (including for a time before diagnosis). The disentangling of the cause and effect relationship between milk yield and disease is not straightforward and has yet to be fully understood. In the instance of detecting cows at increased risk of disease in early lactation, milk yield is of limited value as cows are not lactating in the dry period and therefore it is not possible to record milk yield.

Changes in metabolism in early lactation cause dramatic shifts in milk component ratios, specifically those of milk fat and milk protein (Negussie *et al*., 2013). *Postpartum* lipolysis results in an increasing milk fat percentage while negative energy balance, due to the widened gap between energy intake and requirements, results in a lowered milk protein concentration. Thus, the early lactation period is characterised by a high fat: protein ration (FPR). It has been proposed that in light of the relationship between tissue mobilisation and changes in milk components, FPR may be used as a proxy for energy status, especially in the early lactation period (Heuer *et al*., 1999; Negussie *et al*., 2013). Following an initial rise to peak in the immediate *postpartum* period, FPR has been shown to decline as lactation progresses with a marked increase towards the end of lactation. Negative energy balance and the accompanying increase in FPR is linked to severe metabolic and functional ailments. A FPR of greater than 1.5 was shown to cause increased body condition loss and increased risk of ketosis, displaced abomasum, ovarian cysts, lameness and mastitis (Heuer *et al*., 1999). However, those cows with a FPR >1.5 produced higher milk yields than those with normal FPR but had impaired reproductive performance (Heuer *et al*., 1999). Toni *et al*. (2011) evaluated the prognostic value of early lactation FPR using data from 1498 cows. Cows with a FPR in early lactation greater than 2.0 showed an increase in *postpartum* diseases – retained placenta, left displaced abomasum, metritis and clinical endometritis.

More sophisticated methods of estimating energy balance, and therefore disease risk, have been developed using in-line testing to detect biomarker levels in milk. Chagunda *et al*. (2006) used data collected from in-line testing for BHB to construct a model which allowed the risk of ketosis to be calculated on a scale of 0 – 1 (0 = no risk and 1 = clinical ketosis) for individual cows. In any cases where ketosis risk was elevated, the model output also recommended more frequent testing to allow timely diagnosis. Furthermore, milk electrical conductivity (MEC) has been identified as a potential indicator of health status, with increases in MEC being associated with the onset of intramammary infection (Norberg *et al*., 2004). Maatje *et al.* (1992) used deviations from a running average of MEC for individual cows to indicate mastitis; when combined with milk yield and milk temperature data this model was able to detect all cases of clinical mastitis and 50% of subclinical cases. Additionally, de Mol (1999) suggested that increased MEC may be associated with diseases and disorders other than mastitis. Lukas *et al*. (2009) demonstrated that MEC was increased prior to diagnosis of metabolic and digestive diseases including milk fever, ketosis, left displaced abomasum and retained placenta. In the case of mastitis, significant increases in MEC were detected as early as 10 days pre-diagnosis, highlighting the potential for early detection using this model (Lukas *et al*., 2009). Importantly, milking systems can now record MEC data for individual animals at each milking (Lukas *et al*., 2009).

Milk spectroscopy, using Fourier-transform mid-infrared (MIR-FTIR) is an established technology which has been used for some time in official milk recording schemes to determine the protein, casein, fat, lactose and urea contents of bulk and individual milk samples (De Marchi *et al*., 2014). However, MIR-FTIR can also be used to predict other milk composition traits such as acidity, coagulation properties, amino acid and fatty acid composition (Toledo-Alvarado *et al*., 2021), with a growing body of literature pointing to its value as a means of indirectly measuring a cow's metabolic status due to the interdependence of nutrition, energy balance and milk composition particularly in early lactation. McParland *et al*. (2011, 2012) first investigated the feasibility of using spectral analysis as an indicator of body energy status in Holstein cows in a single herd before evaluating its use across a larger dataset, which proved to be successful in providing useful information on the energy status of cows to dairy farmers (De Marchi *et al*., 2014). Milk MIR was able to predict direct energy balance, body energy content and energy intake with accuracies of 0.47 to 0.69, 0.51 to 0.56 and 0.76 and 0.80, respectively (McParland *et al*., 2012). An advanced application of this technology was investigated by Toledo-Alvarado *et al*., 2021 who established that milk spectral data is associated with fertility outcomes, namely days open although the variance explained by the models they developed was relatively low. In addition, spectral analysis is rapid and low cost and therefore it warrants further studies to assess its predictive ability to use as a technological tool on dairy farms (Toledo-Alvarado *et al*., 2021).

(ii) Body condition score and liveweight

As previously outlined, body energy reserves directly affect disease risk amongst dairy cattle. In a practical setting, body condition score, which estimates body energy reserves by visual assessment may therefore prove to be a useful indicator of health status. Due to the ease of scoring and repeatability of the process, assessing body condition is a widely used management tool (Morin *et al*., 2017).

Kim and Suh (2003) found that cows that experienced a marked loss in body condition had a higher occurrence of metritis and metabolic disease than cows that experienced a moderate loss in body condition score (BCS). The incidence of metabolic disease, including displaced abomasum, milk fever and ketosis, was 32% amongst cows with a marked loss in condition (loss of 1 – 1.5 units of BCS) compared to 2% incidence amongst cows with moderate condition loss (loss of 0 – 0.75 units of BCS). Importantly, high BCS (defined differently in different studies) at calving has been identified as a risk factor for increased lipid mobilisation in early lactation (Heuer *et al*., 1999).

Roche and Berry (2006) demonstrated that over-conditioned cows suffer from greater physiological stress in the early lactation period; cows with a condition score of greater than 3.5 at the time of calving were shown to have suppressed dry matter intake and milk yield in the early lactation period. Studer (1998) had previously found that cows with a high body condition score in the dry period were prone to *postpartum* disease and in particular, fatty liver disease. Shirley (1994), who examined the effect of BCS on the incidence of ketosis and displaced abomasum, reported that *primiparous* cows fed to maintain a high body condition score in the 60 days before calving experienced a high incidence of subclinical ketosis and a 50% incidence of displaced abomasum in the 30 days following calving. Similarly, Cameron *et al*. (1998) identified high BCS as a significant risk factor for displaced abomasum while Gillund *et al*. (2011) found high BCS to be associated with an increased risk of ketosis. Heuer *et al*. (1999) reported that milk fever occurred more often in fat cows whilst Roche and Berry (2006) showed that over-conditioned cows have a 30% greater risk of developing the disease compared to cows with an optimum body condition score. In addition, Collard *et al*. (2000) suggested that a causal relationship may exist between extremely low body energy reserves and immune competence. Roche and Berry (2006) found that cows of below optimum condition were 13% more likely to develop milk fever than cohorts in optimum condition. Conversely, a study by Al Ibrahim *et al*. (2010) showed that cows with a relatively low BCS at calving had reduced milk yields but greater feed intake, improved metabolic status and recovered from BCS loss quicker than those cows of relatively high condition at calving.

The potential for automating body condition score assessment has been investigated and a variety of methods have been proposed. Developments in technology have allowed inexpensive automation of body condition scoring through the application of machine vision (Song *et al.*, 2019). Bewley *et al.* (2008a) demonstrated that image analysis of 2D images could successfully predict actual BCS to within 0.25 points in 89.95% of cases. The use of 3D images to measure BCS have also proved successful; a model developed, by Spoliansky *et al.* (2016) alongside the use of a 3D camera, achieved 91% correct classification of condition within 0.5 points. Although these technologies are useful for collecting measurements and information, little data exists which uses this data to make predictions about the health status of the cow with the exception of 2 studies by Thorup *et al.* in 2018 and 2013 who combined body weight and body condition score data to estimate energy balance. They identified a need for future work to establish a link between energy balance and disease and production outcomes in large data sets.

Automated walk-over weighing systems are also now available which can be used to monitor liveweights of individual cattle. Commercially available walk-over scales, combined with identification technology, can be used to identify and record the liveweight of cattle as they pass over a weighing platform multiple times per day as they enter or exit the dairy or automatic milking system (AMS) for milking (Dickinson *et al.*, 2013). Alwaneh *et al.* (2011) found that results using a walk-over weigh scale were similar to those recorded using conventional weighing techniques and recommended that liveweight be recorded on a daily basis to allow changes in physiological status of individual cows, such as disease or oestrus onset to be detected. For herd-level decisions such as adjusting the herds ration, they recommended a 7-day decision interval to successfully monitor significant changes in cows' liveweight measurements (Alanweh *et al.*, 2013). Clear physiological links between liveweight and metabolic and health status have been demonstrated; if body weight can be measured accurately, changes in weight should reflect the energy status of the cow and therefore could be used as an energy balance indicator (Mantysaari & Mantysaari, 2015). In a study of over 200 cows, Maltz (1997) identified approximately 50% of health problems by bodyweight changes, up to 3 days before milk yield decreased. Weight loss of greater than 12% from calving to nadir liveweight *post*-calving has been shown to significantly reduce the odds of conception at first insemination and in the same study, the odds of insemination on any given day after calving decreased by 21% if a cow lost greater than 7% of its body weight in the first 10 days of lactation (van Straten *et al.*, 2009).

(iii) Activity and behaviour

Currently, activity monitoring in dairy cattle is primarily used for oestrus detection although commercial accelerometer systems increasingly include algorithms to measure time spent eating and ruminating via wearable technology such as collars and ankle-worn pedometers. There is a limited, but growing, body of evidence to suggest that activity monitoring may be of use in detecting production disease. A review examining the use of behaviour to predict and identify ill health in animals by Weary *et al* (2008) *post*ulated that in the event of sickness, behaviours that provide long-term fitness benefits are most likely to decline as animals prioritise critical functions with short-term benefits e.g. regulating body temperature. Work by Moallem *et al*. (2002) confirmed that cows diagnosed with lameness exhibited reduced walking activity but showed no evidence of an association between reduced activity and *post*-calving metabolic disease. However, a study which investigated the value of activity as an indicator of 'fresh cow disorders' found that mean walking activity for sick cows was between 8 and 14 steps per hour less than healthy cows (Edwards and Tozer, 2004). Cows which suffer from sub-clinical ketosis have been shown to visit the feed space less often and spend less time in the area per visit than healthy animals (Goldhawk *et al*., 2009). This, in turn negatively affects DMI, whose maintenance is a crucial aspect of transition cow health; during the period from one week before calving until two weeks after calving, DMI was reduced by 21% in animals with SCK compared to healthy cows (Goldhawk *et al*., 2009). More recently, work by Thorup *et al* (2016) indicated that, when compared to non-lame cows, lame cows are likely to exhibit different feeding behaviour such as increased feeding rate and decreased feeding time.

In addition to "wearable technologies" such as collars and ankle pedometers used to monitor activity, intraruminal radio telemetric boluses which measure temperature and rumen pH are now commercially available. Rumen temperature is an established and effective proxy measure of core body temperature which is useful in the identification of illness, heat stress, general stress and oestrus in dairy cattle (Hicks *et al*., 2001;

Bewley *et al*., 2008b; Ipema *et al*., 2008). Boluses have been shown to detect increases in body temperature associated with bovine respiratory disease, viral diarrhoea and mastitis (Dye *et al*., 2007; Small *et al*., 2008). In the case of mastitis, rumen boluses were able to detect an increase in body temperature associated with experimentally induced udder infection in a more practical manner than vaginal or rectal telemetric measures (Al Zahal *et al*., 2011). Reticuloruminal pH can now also be monitored continuously using commercially available boluses placed in the reticulum which use wireless data transmission, representing significant progress when compared to the original wired electrode lead method described in 1993 (Dado and Allen, 1993; Gasteiner *et al*., 2012;). Continuous measurement allows an increased accuracy in diagnosing subacute ruminal acidosis (SARA) due to the ability to measure diurnal pH changes in contrast to single measurements obtained by rumenocentesis, stomach tube or canula (Humer *et al*., 2017). When using continuous measurement systems, the guidelines suggest that risk of SARA increases when ruminal pH drops below 5.6 for more than 3 hours per day (Plaizier *et al*., 2008) or below 5.8 for more than 6 hours per day (Zebeli *et al*., 2008). Given the advantages of wireless sensors, they represent a considerable step forward in monitoring rumen pH however their limited lifespan and high costs are prohibitive for whole herd use (Humer *et al*., 2017). Based on their study using reticuloruminal boluses on 2 dairy herds and 1 beef herd, Jonsson *et al*. (2019) advised that a minimum of 9 boluses would provide a reasonable estimate of the true mean pH for herds at risk of acidosis, assuming the individual animal variation in pH is similar to that observed in their data.

(iv) Feed and water intake

Feed and water intake, which are affected by appetite, are indicative of health status. The magnitude and longevity of the depression in feed intake throughout the *peripartum* period can be used to identify cows at risk of developing disease. Identification of cows predisposed to partum and *postpartum* health disorders, based on feed intake measures, was proposed by Zamet *et al*. (1979) who demonstrated that voluntary feed intake was, on average, 18 and 20% lower in the peri-partum period. Allen and Piantoni (2013) suggest that the oxidisation of non-esterified fatty acids (NEFA) in the liver causes inappetence, and thus a reduction in food intake. More recently, several studies have traced the effect of feed intake and feeding behaviour in the immediate pre-calving period on the incidence of *post*-calving metritis. Urton *et al*. (2005) reported that cows that developed *postpartum* metritis spent 22 minutes less at the feeding alley per day during the transition period, than cows that remained healthy, with every 10 minute decrease in daily feeding time doubling the risk of metritis diagnosis. Similar results were obtained by Huzzey *et al*. (2007) who found that decreases in feed intake and feeding time were evident two weeks before clinical signs of metritis. Retrospective analysis of disease data has demonstrated rapid daily decreases in feed intake (totalling 10.38kg FW per day) prior to a diagnosis of ketosis (Gonzalez *et al*., 2008). Time spent in the feeder decreased by 19 minutes per day for the 7 days prior to a diagnosis of acute lameness, accompanied by a sharp increase in feeding rate (i.e. these cows consumed food faster) (Gonzalez *et al*., 2008).

Further, water intake is also associated with disease risk. Huzzey *et al*. (2007) established that cows with mild or severe metritis consumed less water than healthy cows during the 3 weeks after calving. Cows that went on to develop mild metritis consumed less water than healthy cows in the 2 weeks pre-calving; raising the possibility of identifying at-risk cows before calving (Huzzey *et al*., 2007). Lukas *et al*., (2008) found that monitoring water intake on an individual cow basis allowed the identification of significant changes which can help detect animals with disease. Their work showed that calving and other health events (including ketosis and milk fever) decreased both feed and water intake. Water intake has also been shown to be associated with reticuloruminal temperature, with water significantly reducing reticuloruminal temperature (Bewley *et al*., 2008b; Ipema *et al*., 2008). Thus, reticuloruminal temperature monitoring, performed by rumen boluses offers an opportunity for water intake to be monitored by proxy (Cantor *et al*., 2018). Results from a study conducted at the University of Kentucky suggested that an algorithm could be developed to predict water drinking bouts for dairy producers using rumen temperature boluses, although caution must be used as both the quantity and temperature of water consumed has affects the baseline temperature (Cantor *et al*., 2018).

(v) Breath analysis

Breath analysis is a further example of a developing technology which may provide a useful source of information on cow health. In 1997 it was demonstrated that acetone concentrations in exhaled breath, measured by gas chromatography and mass spectrometry, were correlated with blood BHB and milk acetone levels and as such displayed potential as a non-invasive method of determining the metabolic status of cows (Dobbelaar *et al.*, 1997). Similarly, further research demonstrated that use of breath samples gave an 89% success rate for the classification of cows as healthy or ketotic and use of a ‘confusion matrix’ correctly predicted 34 out of 38 samples (Elliot-Martin *et al.*, 1997). More recently, the potential of breath analysis to differentiate between healthy cattle and those infected with bovine tuberculosis has been proven (Turner *et al.*, 2012; Ellis *et al.*, 2014). Although ample evidence exists which outlines the potential of breath analysis to distinguish between healthy and sick animals, challenges remain in developing sampling devices which take representative and reproducible breath samples from cattle safely, with minimal stress to the animal and which could be used on commercial dairy farms (Turner *et al.*, 2012).

(vi) Automatic milking systems

Automatic milking systems (AMS) represent one of the most recent technological advances in the dairy industry and in 2009 an estimated 8000 farms worldwide, mainly in northern Europe and Canada, had adopted AMS (de Koning, 2010). By 2020, AMS manufacturers estimated that this had risen to 50,000 farms and forecast that by 2025, 50% of dairy cows in north-western Europe will be equipped with AMS (Cogato *et al.*, 2021). AMS are equipped with sensor technology and integrated data management systems, and as such a key advantage of AMS adoption is the availability of daily cow-level data that are collected (De Koning, 2010; King & DeVries, 2018). The adoption of AMS allows for less labour-intensive collection of data from all the sources previously outlined in this section – milk yield and quality, body condition score and bodyweight, behaviour and activity, feed and water intake and breath analysis and as such represents a very important opportunity to allow for improved disease detection. As a result, Tse *et al.* (2017) reported that 80% of producers found illness detection to be easier when they adopted AMS technologies than before. Currently the main variables routinely recorded by AMS in commercial settings are milk yield, rumination time, activity, and body weight, the value of which as indicators of health status has already been outlined (King & DeVries, 2018).

1.2.3.2 Data analysis – predicting and modelling disease risk

Although precision livestock farming (PLF) technologies, such as those previously described, enable the collection of more precise data, several key challenges exist in "translating" the large volume of data generated from these new technologies into timely and useful information for producers (King & DeVries, 2018; Rojo-Gimeno *et al.*, 2019). Firstly, currently most data and information sources are fragmented and difficult to use, meaning that the full potential of the data is not being exploited (Fountas *et al.*, 2015). Secondly, from a mathematical perspective, the data must be in a form which can serve as inputs for software that yields estimations or decisions as outputs using validated models and algorithms (Maltz and Metz, 1994). Thirdly, data handling techniques, such as smoothing, which allow differentiation between changes which are of physiological significance and those which are normal daily fluctuations must be adopted (Maltz & Metz, 1994). In addition to these challenges, the vast majority of work in the field of dairy cow health and disease risk has focused on identifying individual risk factors associated with disease rather than developing predictive models that accurately estimate whether a disease is present or is likely to develop (Wisnieski *et al.*, 2019a). Therefore, the ability to successfully model disease risk relies on (1) integration of data from various monitoring systems, (2) data being stored in such a way that it can be used in algorithms or statistical models, (3) techniques being developed to successfully distinguish between truly "abnormal" events and normal fluctuations and (4) progressing from the identification of risk factors to developing truly predictive models.

1.2.4. Literature Review Summary

In summary, there are many potential disease indicative risk factors which can feasibly be recorded on farm at an individual cow level. However, there remains a need for work to identify which of these pieces of data are most closely related to disease risk and to assess their potential to identify cows at risk of disease, particularly in the transition period. This presents a unique challenge, as during the dry period, monitoring is not as intensive and much of the data discussed above may be unavailable. Current levels of disease and physiological stress among modern dairy cattle are unacceptably high and a focus on further increasing milk yield will exacerbate this problem. The transition period represents the climax of physiological stress in the lactation-gestation cycle due to the series of physical, metabolic and hormonal changes orchestrated to occur around calving, meaning that disease incidence is highest in early lactation. The true extent of the effects of early lactation disease on health and productivity must be quantified in modern dairy cattle, as much of the classic work in this field is now outdated (e.g. Kossaibati and Esslemont, 1997).

To protect animal welfare and minimise the negative financial implications of disease, it is necessary that sick animals are identified and treated promptly. To allow the development of systems that can monitor the health status of individual cows and to aid in the identification of at-risk animals it is necessary to determine which physiological or production traits are indicative of health status. Potential indicators should be measurable without the use of invasive procedures and without the need for capital investment in specialist equipment. Therefore, extraction and identification of health status indicators from data which is currently recorded on farm should be investigated to establish their value and potential usefulness in disease detection systems. Current research work being undertaken to specifically predict transition cow disease occurrence will be reviewed in Chapter 4.

1.3 Study approach

Due to the sustained level of disease in high-producing dairy herds at a time of increasing herd size, it is important to explore methods of aiding early detection of disease in the context of increasing automation and the adoption of precision livestock farming practices. This project aimed to identify indicators of disease, measured in individual cows, and to evaluate their potential ability to identify cows at risk of developing disease in early lactation. This study sought to exploit the potential of on-farm data by only using data collected from devices which could realistically be installed on commercial dairy farms soon. In doing so, the study intended to identify which aspects of routinely recorded on-farm data were reflective of individual cow health status.

1.4 Study objectives

The objectives of this thesis were:

- To quantify the effect of early lactation disease on milk yield, fertility and culling

Initial research was conducted to establish the effect of early lactation disease on measures of productivity. Analysis of variance was used to compare mean daily milk yield, peak milk yield, days to peak yield, days to first heat, days to first service, number of inseminations per conception and calving interval between four groups of cows which were classified according to health status in early lactation. Eventual reasons for culling for cows in each health group were also investigated.

- To identify candidate indicators of early lactation disease, recorded at the end of lactation and throughout the dry period, which were different between healthy and non-healthy cows

To allow for further development of automated disease detection aids, the identification of potential disease indicators is required. The objective of this study was to identify traits recorded throughout the dry period which were significantly different between cows that went on to develop different diseases in the subsequent lactation. Data from the dry period preceding the lactation in which health classification was performed was extracted for cows in each of the four health groups. Data underwent extensive preliminary analysis to identify possible disease indicators which could be derived from measurable traits. Potential indicators were compared between each of the four health groups.

- To assess the potential of disease indicators identified in this study for use in predictive models to distinguish between healthy and sick cows, for future inclusion in health monitoring aids.

Potential disease indicators identified in the earlier stages of the current study underwent further analysis to assess their ability to successfully discriminate between healthy and sick cows. Additionally, the relationships between potential indicators were evaluated using correlation analysis before single and multi-variable models were constructed.

Chapter Two

The effect of early lactation disease on dairy cow productivity

2.1 Introduction

Maximisation of farm profits is an important goal of all commercial dairy farmers (Renkema and Stelwagen, 1979; Chamberlain, 2012). Historically, attempts to maximise profits focussed on increasing milk output per cow with little consideration for health and reproduction (Ingvartsen, 2006). However, due to the antagonistic genetic relationship between milk production and traits such as fertility and disease, selection resulted in an undesirable increase in health and fertility problems (Zwald *et al.*, 2004, Ingvartsen, 2006; Farm Animal Welfare Council, 2009). This concomitant increase in health and fertility problems may negate the financial benefits of genetic selection for milk yield by simultaneously reducing farm outputs and increasing inputs (Ingvartsen, 2006). Therefore, quantifying the effect of disease on individual cow productivity is essential in understanding its economic impact on dairy herds.

In general, health disorders lead to a temporary decrease in milk yield around the time of disease, the magnitude of which is disease specific (Barielle *et al.*, 2003). However, disease also exerts negative effects on lactation and lifetime milk production; cows diagnosed with an LDA were found to have lactation losses of between 400 and 800 kg (Fourichon *et al.*, 1999). More recently, similar results have been obtained by Carvalho *et al*. (2019) who reported that cows with an incidence of clinical disease in the first 21 days of lactation had a 305 day yield which was reduced by 410kg compared to cows with no clinical disease in the same period. However, it is critical to acknowledge that it can be difficult to disentangle cause and effect in health and production studies. In addition to disease-mediated drops in milk yield, high milk yield is itself a risk factor for several production diseases, including ketosis and hypocalcaemia (Ostergaard and Grohn, 1999; Rajala-Schultz *et al.*, 1999). Thus, it may be expected that disease incidence in the highest yielding cows in any given population will be higher than in their lower-yielding contemporaries.

Furthermore, reproductive performance is linked to health immediately before and after calving and consequently early lactation disease has a negative impact on fertility (LeBlanc, 2010). Retained placenta, metritis and subclinical ketosis have been associated with a delayed resumption of oestrus and a reduction in conception rate at first service (Fourichon *et al*., 2000; Raboisson *et al*., 2014). In addition, disease affects overall production system productivity via the increased culling rate it causes. Mastitis within the first 30 days of lactation is associated with a significantly increased culling risk (Pinedo *et al*, 2014).

Few studies exist which solely examine the effect of early lactation disease on short- and longer-term measures of productivity. Studies examining the effect of disease on milk production are often difficult to interpret due to the confounded nature of the relationship between milk production and disease. Therefore, health and production studies often provide conflicting results. Except for Hostens *et al*. (2012), most studies investigating the effect of disease on milk yield are dated. The current study offers an opportunity to examine the effects of disease on production, fertility, and longevity in a single herd over an extended period where all aspects of management are controlled and recorded.

2.2 Hypothesis and Objectives

The hypothesis of the current study was that cows which developed production diseases in early lactation would have reduced productivity compared to cows which remained healthy. Further, it was hypothesised that different production diseases would have different effects on productivity. This was tested by analysing data from cows of known *post*-calving disease status. The objective of this study was therefore to quantify the effects of production disease on measures of productivity in dairy cattle. Specifically, the effect of production disease on milk production, fertility and culling were investigated under the null hypothesis that there are no differences in these parameters between groups with different health/fertility outcomes.

2.3 Materials & Methods

2.3.1 Data source

Data were obtained for this study from the Langhill herd of Holstein-Friesian cattle at Scotland's Rural College's (SRUC) Dairy Research and Innovation Centre, Crichton Royal Farm, Dumfries. Data used were from a period of eight years, from November 2003 to September 2011 when cattle were on a long-term 2x2 factorial experiment which investigated the interaction between genotype and environment.

2.3.2 Feeding Regimes

Animals were maintained in two feeding regimes: low forage (LF) and high forage (HF). Cows in the LF system were housed continuously and fed a total mixed ration (TMR) composed of 40-45% forage on a dry matter (DM) basis (Chagunda *et al.*, 2009). Cows in the HF system were grazed when grass growth and ground conditions permitted. Cows grazed for three periods each day on perennial ryegrass swards when compressed grass height exceeded 10cm. Grazing periods were reduced to two per day and one per day when compressed grass heights fell below 10 and 7cm, respectively. Table 2.1 provides details of the grazing periods for HF cows throughout the study. When housed, HF cows were fed a TMR composed of 70-75% forage on a DM basis. Housed rations for both regimes comprised a mix of three home grown forages (ryegrass silage, whole crop wheat alkalage, and whole crop maize silage), purchased concentrate blend and minerals. Representative daily TMR formulations for lactating cows during housing for each regime are presented in Table 2.2.

Table 2.1: Yearly grazing start and end dates for the study period with number of days with less than six or greater than six hours grazing for cows under High Forage regimes

Year	Grazing start date	Grazing end date	Total days <6 hours grazing	Total days >6 hours grazing	Total grazing days
2003	07/04/2003	08/10/2003	17	165	182
2004	22/03/2004	05/10/2004	31	164	195
2005	31/03/2005	31/10/2005	72	140	212
2006	13/04/2006	15/11/2006	39	175	214
2007	07/04/2007	27/10/2007	38	153	191
2008	04/04/2008	23/10/2008	47	153	200
2009	01/04/2009	18/11/2009	48	181	229
2010	10/04/2010	25/11/2010	47	180	227
2011	26/03/2011	26/11/2011	52	191	243

Table 2.2: Total mixed ration (TMR) components expressed as percentages (%) of the total formulation offered to lactating cows under Low Forage and High Forage regimes on a fresh weight basis

TMR Component	Low Forage (%)	High Forage (%)
Ryegrass silage	27.0	45.0
Urea-treated wholecrop wheat	9.0	15.0
Maize silage	9.0	15.0
Purchased concentrate/blend	53.9	24.2
Minerals	1.1	0.8

The LF diet was formulated to provide, on average, 11.7 megajoules (MJ) and 180 grams (g) of crude protein per kilogram (kg) of DM. The HF diet provided, on average, 10.8 MJ and 171 g of crude protein per kg of DM with an average DM intake of 23.4 kg/cow/day. Nutritional composition of the TMRs shown is presented in Table 2.3. Where possible, female progeny were assigned to the same feeding regime as their dam.

Table 2.3: Descriptive statistics for feed characteristics obtained from analysis of feed sampled weekly over the full study period (SRUC Analytical Services Department). Where NCGD = neutral cellulase gammanase digestibility, an enzyme-based technique used to estimate the digestibility of feed. Data taken from (Ross, 2014).

		Low Forage		**High Forage**	
Characteristic	Unit	Mean	s.d.	Mean	s.d.
Dry Matter content	g/kg	426	47.8	349	43.7
Crude Protein content	g/kg DM	180	13.5	171	12.2
Digestibility (NCGD)	g/kg DM	852	34.4	757	34.9
Metabolisable Energy	MJ kg/DM	11.7	0.44	10.8	0.65

2.3.3 Genetic lines

Within each feeding regime, animals belonged to one of two genetic lines. Control (C) cows were bred to be of average UK genetic merit for milk fat and protein production and Select (S) cows represented the top 5% of UK genetic merit for the same traits (Pryce *et al.*, 1999). To maintain these genetic lines, S cows were sired by bulls with high predicted transmitting ability (PTA) for milk fat plus protein yield and C cows were sired by bulls of average UK genetic merit for milk fat plus protein yield. The factorial nature of the long-term experiment meant that 4 sub-herds were maintained for the duration of the study, namely, high forage control (HFC), high forage select (HFS), low forage control (LFC) and low forage select (LFS). The Predicted Transmitting Ability (PTA) and Profitable Lifetime Index (PLI) for the genetic lines is presented in Table 2.4. The PLI value represents the additional profit each animal is expected to return over her lifetime compared with an baseline of £0, which is re-set every 5 years. It is important to note that the PTA and PLI figures presented are calculated on a current year basis (April 2021) i.e. the PLI of the cows in the study is in comparison to the 2021 PLI baseline. Thus, all PTA and PLI figures are negative, as the baseline for each of these traits has increased since the time of the study. Cows were transferred out of the systems study at the end of their third lactation provided a replacement heifer was available to maintain the group sizes at approximately 50 cows. If at all possible, animals were retained within the same diet group as the dam; then as far as possible every step was taken to ensure average PLI was similar between diet groups, although sire was not considered at this stage.

Table 2.4: Mean genetic index figures for control and select cows for the period 2003 – 2011 calculated on a current year basis (comparison to baseline PTA and PLI values as of April 2021)

Genetic Line	Genetic Index	Mean	S.E.M.
Control	PTA Milk (kg)	-610	11.5
	PTA Milk fat (kg)	-24	0.3
	PLI (£)	-335	4.4
Select	PTA Milk (kg)	-105	11.8
	PTA Milk fat (kg)	-3	0.4
	PLI (£)	-131	7.6

2.3.4 Herd Management

Cows were milked three times daily and were subject to the same general management procedures (e.g. bedding, lighting, foot-trimming routines) under the responsibility of the same staff. Cows were only physically separated based on feeding regime – S and C cows on the same feeding regime were housed together. Cows calved all year round. Production details of each of the 4 sub-herds across all lactations between 2003 and 2011 are presented in Table 2.5.LFS cows were the highest producing in terms of milk yield and energy corrected milk yield with mean 305-day yields of 35.5 kg/day and 34 kg/day respectively. HFC cows produced the least volume of milk and had the lowest proportion of milk fat and milk protein, meaning that they had the lowest energy corrected milk yield on a 305-day yield basis. LFC and HFS cows were of similar body weight. LFS cows had the highest mean body weight; however, LFC cows had the highest mean condition score and body condition score.

2.3.5 Fertility Management

Heifers and cows were served by artificial insemination (AI). Maiden heifers were inseminated for the first time at approximately 13 months old to ensure first calving at around 24 months of age. After each calving, cows were inseminated at the first observed heat after day 42 of lactation. In general, cows were served up to 7 times per breeding period before being removed from the herd. Cows which did not display oestrus by day 42 of lactation were examined by a veterinary surgeon. All reproduction events were recorded and included first observed oestrus cycle, dates and number of services and dates and results of pregnancy scanning. An all year-round calving policy was maintained throughout the entire study period.

Table 2.5: Details of production system (genetic line x feeding regime) - mean 305-day milk yields, mean 305-day energy corrected milk yield, milk fat, milk protein, body weight, metabolic body weight and body condition score across all lactations between 2003 - 2011

	System							
	Low Forage Control		**Low Forage Select**		**High Forage Control**		**High Forage Select**	
	Mean	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
Energy corrected milk yield (kg/day)	28.0	7.70	34.0	8.17	23.0	6.74	27.0	7.38
Milk yield (kg/day)	30.5	8.93	35.5	9.36	23.9	7.43	27.1	7.88
Milk fat (kg/day)	1.1	0.33	1.3	0.35	0.9	0.28	1.1	0.32
Milk protein (kg/day)	0.9	0.25	1.2	0.27	0.7	0.22	0.8	0.23
Body weight (kg)	623.8	75.12	636.5	76.48	598.8	77.19	626.7	79.84
Metabolic body weight* (kg)	124.6	11.28	126.5	11.45	120.8	11.72	125.1	12.04
Body condition score	2.3	0.40	2.2	0.41	2.2	0.36	1.9	0.37

*Where Metabolic body weight = Body weight $^{0.75}$

2.3.6 End of Lactation and Dry Cow Management

Cows were dried off at approximately 7 months gestation. At the time of dry-off, all cows were treated with a long-acting intra-mammary dry cow antibiotic, and vaccinated using Rotavec™ (MSD Animal Health, Milton Keynes, United Kingdom) for the subsequent prevention of diarrhoea in the newborn calf. Throughout the dry period cows underwent weekly foot bathing using a copper sulphate solution. From dry-off until 3 weeks before predicted calving date, dry cows were housed in cubicles and fed a straw based ration which was delivered daily. Cows were moved to loose house straw pens three weeks before predicted calving date, where they were maintained in groups of between 10 and 14 cows. For the remaining three weeks of the dry period, cows were fed a transition diet which consisted of one third of the lactation ration for their respective production group (i.e. LF or HF), supplemented with straw. Details of the TMR dry cow diet and respective transition diets are given in Table 2.6.

Table 2.6: Total mixed ration (TMR) components expressed as percentages (%) of the total formulation offered to dry cows (from dry off until three weeks before predicted calving date) and to transition cows (within three weeks of predicted calving date) under Low Forage and High Forage regimes on a fresh matter basis

	Lactation Stage		
TMR Component (%)	**Dry**	**Low Forage Transition**	**High Forage Transition**
Ryegrass silage	30.0	17.0	27.0
Urea treated wholecrop wheat	10.0	6.0	9.0
Wholecrop maize silage	10.0	6.0	9.0
Purchased concentrate/blend	4.1	32.0	14.0
Wheat straw	45.0	39.0	41.0
Minerals	0.9	1.0	1.0

Straw is a major constituent of each of these rations, however the % of concentrates included is markedly different between the diets. The Low Forage diet includes 32% purchased concentrates while the High Forage diet includes 14%. After calving, cows were re-introduced to the milking herd at the earliest opportunity, usually within 24 hours.

2.3.7 Data Collection

Milk yield, peak flow rate and cow stall position were recorded at each milking. Individual representative milk samples were taken on a weekly basis to be analysed for fat, protein, and somatic cell count. On the day of recording, milk was taken from each cow at each milking (morning, afternoon and night). All milking cows were weighed 3 times daily on leaving the milking parlour by means of a walk over weigh scale (Insentec BC, Marknesse, The Netherlands). Individual cows entered the weigh scale separately and were required to stand still to obtain an accurate body weight. Cows were weighed once weekly throughout the dry period. Body condition score was assessed and recorded weekly throughout the lactation and dry periods by experienced, trained assessors following standardised protocols. Assessors alternated every week to reduce the effect of operator bias, and regular re-training was provided by the same veterinary surgeon for the entire period of study. Body condition scoring was recorded on a 0 to 5 scale to 0.25 units as per Lowman *et al.* (1973). BCS data collected throughout the long-term genetics and environment study has been used previously in a variety of analyses (Randall *et al*., 2015; Chiumia *et al*., 2013). Calving ease, calf birth weight, number of calves and calf sex were recorded at the point of calving. Calving ease was classified according to degree of assistance (farm staff or veterinary staff), calf presentation (normal presentation or malpresentation) and delivery method (natural or caesarean). All reproduction events were recorded in the herd database and included date of first observed oestrus cycle after calving, dates and number of insemination(s) and dates and results of pregnancy scanning. Fertility measures were calculated using calving dates and insemination dates and included: calving interval (CI), days to first heat (DFH), days to first service (DFS), number of services per conception (SPC), first service conception rate, 100 day in calf rate and overall conception rate. Culling date and the primary reason for culling were recorded for each cow at the point of culling, however in most cases this occurred after cows were removed from the experiment as cows were only maintained in the long-term study until completion of lactation 3. Culling data for after lactation 3 is therefore not complete.

All disease diagnoses were performed by a veterinary surgeon or a senior stockperson and recorded in the herd database. Standard operating procedures for the identification of diseases were in place throughout the study period. Senior herdsmen were responsible for diagnosing cases of lameness, mastitis and retained placenta. Milk samples were taken from all mastitic cows and analysed to identify causal bacteria via bacteriology. Suspected cases of metritis, ketosis, hypocalcaemia, hypomagnesaemia and left displaced abomasum were identified by stock workers prior to formal diagnosis by a veterinarian. Throughout the entire study period, the same senior veterinary surgeon was responsible for the strategic health management of the herd. All health events were diagnosed by a veterinarian or by a trained staff member who was required to follow a standard protocol to identify and treat illness. All incidences of disease were recorded by indicating the disease present and the date of diagnosis. Foot trimming and routine vaccinations were recorded as routine health treatments.

2.3.8 Data Handling

Cow-lactations were used as the experimental unit throughout the analysis. Four hundred and eighty cow-lactations, from 399 individual cows, moving between lactations 1 and 2, and 2 and 3 were used in this study. Distribution of these cow-lactations across production system and parity are presented in Table 2.8 (Results). Lactation 1 data was excluded from this analysis due to the differences in physiology and management between maiden heifers and dry cows. In terms of physiology, cows which lose body energy after calving return to a positive energy balance between 40 and 80 days *postpartum* (Coffey *et al*., 2002). However, in first lactation heifers, cumulative body energy losses are not recovered until around day 200 *postpartum* (Coffey *et al*., 2002). In addition, heifer housing and feeding practices were significantly different to those experienced by dry cows - primarily due to the absence of a dry period prior to the birth of the heifer's first offspring.

2.3.9 Classification of cow-lactations

All cow-lactations were assigned to 1 of 4 groups based on disease incidence in the first 30 days of the on-going lactation. These groups were no clinical disease, reproductive, mastitis and metabolic. To qualify for inclusion in 1 of the 4 health categories, cow-lactations were subject to the criteria presented in Table 2.7. There was inconsistency in the diagnosis and reporting of clinical mastitis which did not allow its inclusion in the current study although some cases of clinical mastitis will be included in the subclinical group, based solely on somatic cell count.

Table 2.7: Classification criteria for each health category used for each cow-lactation

Health group	**Definition**
No clinical disease	No clinical disease diagnosis and somatic cell count less than 250,000 cells/ml in the first 30 days of lactation
Subclinical mastitis	At least one recorded somatic cell count greater than 250,000 cells/millilitre in the first 30 days of lactation
Reproductive	Clinical cases of retained placenta (failure to expel foetal membranes within 24 hours of calving) - diagnosed by farm staff OR clinical cases of metritis (abnormally enlarged uterus, vaginal discharge and systemic illness/fever with a temperature >102.5^{o}F) – diagnosed by veterinary surgeon.
Metabolic	Clinical cases of hypocalcaemia (low blood calcium levels, lack of rumen activity and recumbency), hypomagnesaemia (low blood magnesium levels, excitability/hypomagnesaemic tetany), left displaced abomasum (sudden decrease in milk yield, reduced feed intake secondary ketosis) and ketosis (decreased concentrate intake, lethargy and abnormal behaviour) - diagnosed by veterinary surgeon

Cows diagnosed with more than 1 disease were assigned to the health group of the most severe disease (Goff, 2006). The number of cow-lactations with multiple disease diagnoses in the first 30 days of lactation accounted for 0.2% of the cow-lactation records used in this study. 3 cow-lactations had a record of subclinical mastitis and a reproductive disorder (classified as reproductive disorder), 4 had a record of mastitis and a metabolic disorder (classified as metabolic) and 5 had a record of reproductive and metabolic disorder (classified as metabolic).

2.3.10 Statistical Analysis

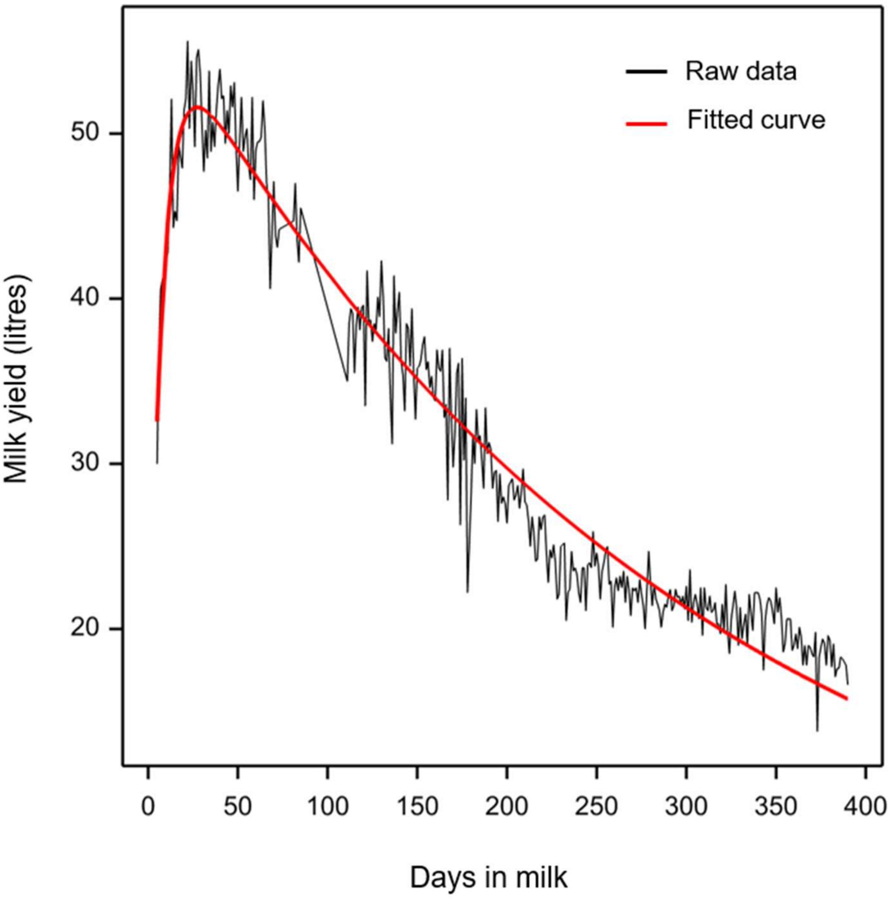
Data were extracted from a SQL database for analysis in Microsoft Excel and Genstat. Data extracted were: cow identity, genetic group, feeding group, lactation number, date of dry off, date of calving, daily milk yield for the on-going lactation, date of first observed heat, date of first insemination, date of next calving, date of culling and primary culling reason.

Daily milk yield data were collated for each cow-lactation; any lactation which included fewer than 50 observations was excluded from the analysis. Curves were fitted to the raw data using a method adapted from Ehrlich *et al.* (2011) to summarise the magnitude and shape of each individual lactation curve.

$milk\ yield = a\left(1 - \frac{e^{\frac{c-t}{b}}}{2}\right)e^{-dt}$ where *t* is days in milk and *a, b, d* are positive

The four resulting estimated parameters (*a, b, c* and *d)* describe the overall lactation scale (*a*), the steepness of the increase in milk production *post*-calving (*b*), the offset in time between calving and maximum growth rate of productive capacity (c) and the loss of productive capacity throughout lactation (*d*). From these parameters, the summary statistics over each curve for time to peak yield, peak yield and cumulative 305-day milk yield were calculated. Figure 2.1 shows an example fitted lactation curve generated from the raw milk yield data for an individual cow-lactation.

Figure 2.1: Lactation curve for an individual cow-lactation (*a* = 58.01, *b* = 7.933, *c* = 3.783, *d* = 0.003) where 305-day yield is 10735 litres, time to peak yield is 27 days and peak yield is 51.39 litres. $R^2 = 95.45\%$



Linear mixed models were used to determine the effect of health group on 305-day yield, peak yield and time to peak yield, as derived from the lactation curves. Production system, parity and calendar year were also included as fixed effects as it had been observed that these had an influence on milk yield (See Figures 2.2 and 2.3). Individual cow was included as a random effect. The following statistical model was used:

$$y_{iklmno} = \mu + S_i + H_k + P_m + A_n + C_o + \varepsilon_{iklmno}$$

where y was the production trait under investigation, μ is the overall mean; S_i is the fixed effect of dairy production system (LFC, LFS, HFC, HFS); + H_k was the fixed effect of health group (1,2,3,4) ; P_m was the fixed effect of parity (2 or 3); A_n was the fixed effect of calendar year; C_o was the random effect of individual cow; $\varepsilon_{ijklmno}$ was the random error term. This analysis was performed twice, once including all data generated from the fitted lactation curves and then where data were excluded when the percentage variation explained less than 20% of the raw data from the fitted.

Figure 2.2: Individual cow fitted 305-day lactation curves according to genetic line

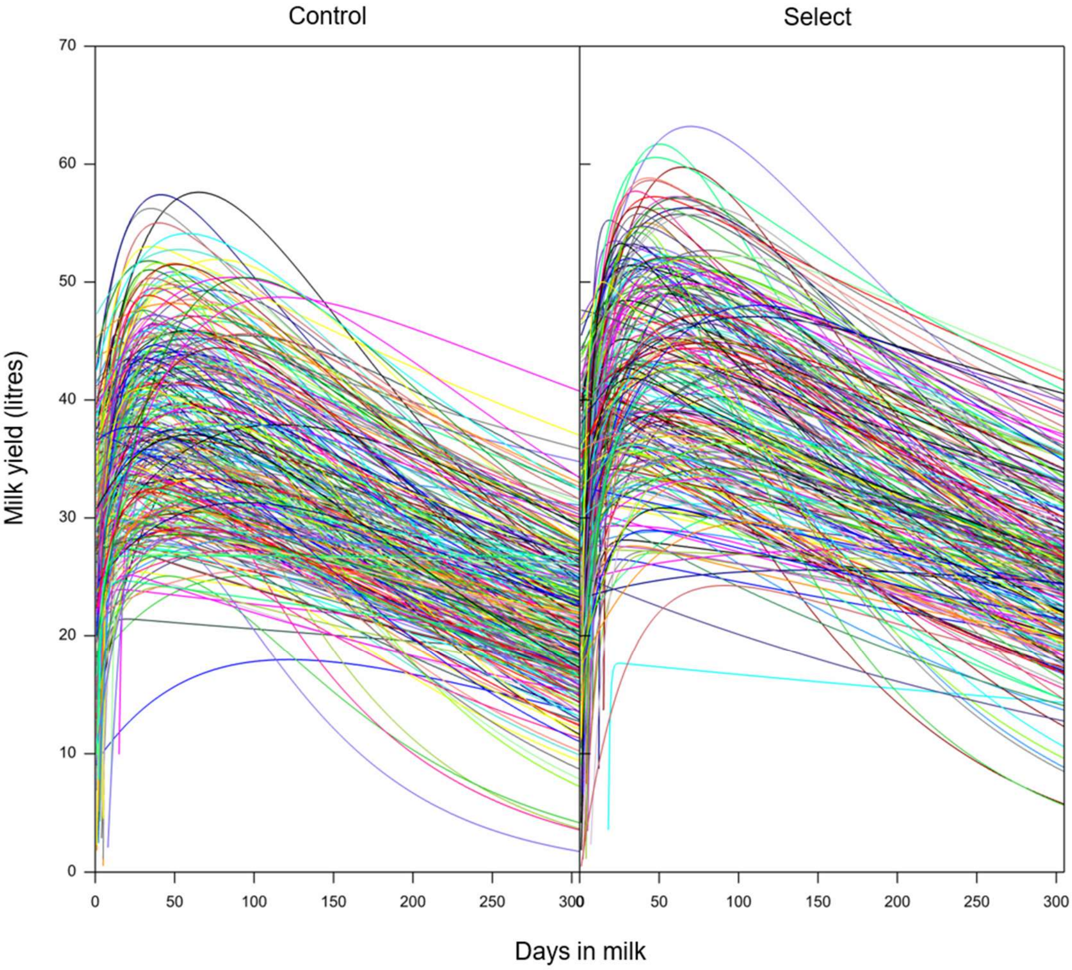
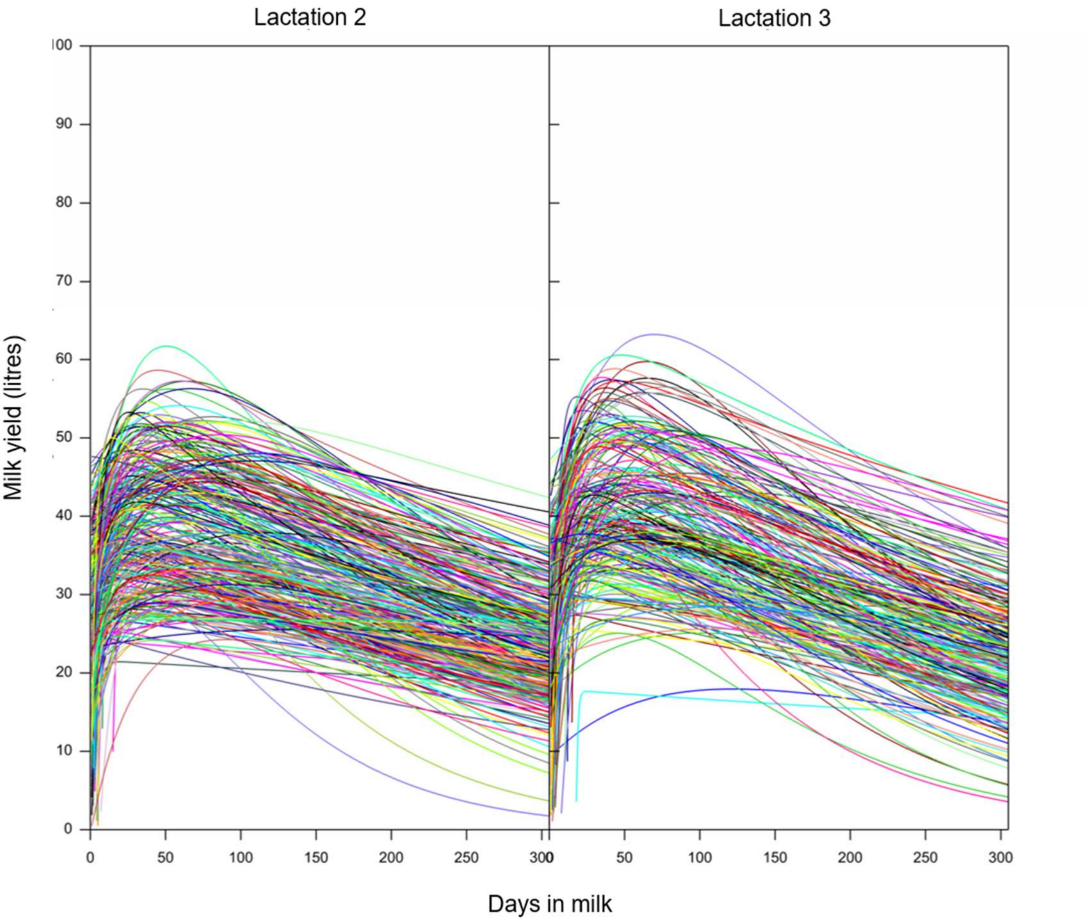


Figure 2.3: Individual cow fitted 305-day lactation curves according to parity



Similarly, linear mixed models (see above) were used to determine the effect of health group on days to first heat (DFH), days to first service (DFS), number of services per cow-lactation and calving interval (CI). Conception rate to first service, 100 day in-calf rate and overall conception rate were calculated from the recorded fertility data. This was analysed by means of a series of Chi-squared permutation tests to test for differences in conception rates according to health group. Due to the nature of this type of test, once significance has been established there are no *post-hoc* tests to determine where the between-group differences exist.

The effect of health group on eventual cull reasons was also analysed by means of a series of Chi-squared permutation tests to test for differences between the proportion of cows culled for particular reasons according to health group.

2.4 Results

2.4.1 Descriptive Statistics

Diseases recorded in this study were all naturally occurring. Their distribution across the four production systems and parities, alongside the health group sizes are presented in Table 2.9. A total of 335 cow-lactations had no record of clinical disease or elevated SCC in the first 30 days of lactation and were classified as having no clinical disease. One hundred and forty-seven cow-lactations were identified as having disease in the first 30 days of lactation. Fifty-three cow-lactations included a diagnosis of subclinical mastitis, 77 had a diagnosis of a reproductive disorder and 17 had a diagnosis with a metabolic disorder. Reproductive disorders (metritis and retained placenta) were the most diagnosed diseases across all production groups and in both parities. LFS cows had the highest total disease incidence (37%). Metabolic disease was most prevalent in the HFS group and in parity 3 cows. Except for early lactation mastitis, cows in parity 3 had a higher overall disease incidence than those in parity 2. Genetic indexes (PTA for milk yield, PTA for milk fat and PLI) were not found to be significantly different between health groups (Table 2.8), although numerically the subclinical mastitis group had the highest PTA for milk yield (kg). It is important to note that these indexes are reported on a current year basis (April 2021), so the absolute values have been adjusted over time since the study period ended (2003 -2011) but the differences will have remained the same.

Table 2.8: Mean genetic indexes (PTA milk, PTA milk fat and PLI) for each health group for the period 2003 – 2011 calculated on a current year basis (as of April 2021)

	PTA Milk (kg)		PTA Milk fat (kg)		PLI (£)	
Health group	**Mean**	**s.e.m**	**Mean**	**s.e.m**	**Mean**	**s.e.m**
No clinical disease	-380	9.6	-14	0.3	-235	4.9
Subclinical mastitis	-348	27.9	-14	0.9	-251	14.3
Reproductive	-393	20.9	-15	0.6	-257	10.7
Metabolic	-355	40	-13	1.2	-272	20.6

Table 2.9: Number of cow-lactations assigned to each health group by system and parity according to criteria in Table 2.5

	Production System				**Parity**		
Disease Classification	**Low Forage Control**	**Low Forage Select**	**High Forage Control**	**High Forage Select**	**2**	**3**	**Total group size (n)**
No clinical disease	93	63	106	73	203	132	335
Subclinical mastitis	14	16	13	10	25	28	53
Reproductive	20	19	19	19	42	35	77
Metabolic*	4	3	3	7	5	12	17
Total disease (n)	38	38	35	36	72	75	147
Total disease (%)	29	37	24	33	26	36	43
Total cow lactations	131	101	141	109	275	207	482

2.4.2 Milk yield

Results from the analysis of milk yield will be presented from 2 sub-sets of data. Firstly, results are provided from the analysis of all data generated from the fitted lactation curves irrespective of the fit of the curves to the raw data. Secondly, results are presented from the same analysis of a smaller dataset which excluded data from the fitted lactation curves where the percentage variation in the raw data explained by the fitted curve was less than or equal to 20%.

(i) Data from all fitted lactation curves

There were significant differences in mean peak yield between health groups ($p<0.001$) (Table 2.10). Cows with no clinical disease and those with subclinical mastitis had significantly higher peak yields (40.7 and 40.1 litres, respectively) compared to cows with reproductive or metabolic disease. Numerically, cows diagnosed with metabolic disease had the lowest peak yield (37.6 litres) but this was not significantly different to the peak yield of cows with reproductive disease (38.3 litres).

Table 2.10: Mean peak milk yield and days to peak milk yield for each health group from all fitted lactation curves

Health group	**Mean peak yield (litres)**	**s.e.m**	**Mean days to peak**	**s.e.m**
No clinical disease	40.7^{a}	0.28	45.4^{c}	1.53
Subclinical mastitis	40.1^{a}	0.80	41.5^{c}	4.26
Reproductive	38.3^{b}	0.56	50.3^{b}	2.96
Metabolic	37.6^{b}	1.26	61.1^{a}	6.67

*Different superscripts within column indicate significant differences between means ($p<0.05$)

Time to peak yield was also significantly different between health groups ($p=0.04$). Days to peak yield were similar between cows with no clinical disease and those with subclinical mastitis, however cows with reproductive or metabolic disease had significantly longer intervals between calving and peak yield. Cows with metabolic disease took, on average, 15.7 days longer to achieve peak yield than cows with no clinical disease. Cows diagnosed with reproductive disease achieved peak yield approximately 5 days later than those with no clinical disease and 9 days later than cows with subclinical mastitis.

305-day yields were also significantly affected (p=0.002) by health group (Table 2.11). Cows with no clinical disease in the first 30 days of lactation had the highest 305-day yield (mean = 9968 litres, s.e.m = 72.4) although this was not significantly different to the yield of cows with subclinical mastitis (mean = 9710, s.e.m = 217.2) or that of cows with metabolic disorders (mean = 9544, s.e.m = 338.9). Cows with reproductive disease had significantly lower 305-day milk yields (mean = 9344 litres, s.e.m = 151.0) than cows with no clinical disease and cows with subclinical mastitis; they yielded, on average, 624 litres less than cows with no clinical disease. The 305-day yield of cows diagnosed with metabolic disorders was not significantly different to the yields of cows from any other health groups, owing to the large standard error of the mean associated with this group.

Table 2.11 Mean 305-day milk yield (litres) for each health group from all fitted lactation curves

Health group	305-day yield (litres)	s.e.m
No clinical disease	9968^{a}	72.4
Subclinical mastitis	9710^{a}	217.2
Reproductive	9344^{b}	151.0
Metabolic	9544^{ab}	338.9

*Different superscripts within column indicate significant differences between means (p<0.05)

(ii) Data from fitted lactation curves where percentage variation explained is greater than 20%

Cows with no clinical disease had significantly higher peak yield than cows of all other health groups (mean = 41.8 litres, s.em. = 0.38) (p=0.003) (Table 2.12). Cows diagnosed with metabolic disease had the lowest peak yield (mean = 37.6 litres, s.e.m

= 1.59). However, this was not significantly different to the peak yield of cows with either subclinical mastitis or reproductive disorders. Time to peak yield was not significantly affected by health group.

Table 2.12: Mean peak milk yield and days to peak milk yield for each health group from fitted lactation curves with percentage variation explained of greater than 20%

Health group	Mean peak yield (litres)	s.e.m	Mean days to peak	s.e.m
No clinical disease	41.8^{a}	0.38	42.5	1.84
Subclinical mastitis	39.9^{b}	1.08	41.7	5.25
Reproductive	39.3^{b}	0.84	48.0	4.09
Metabolic	37.6^{b}	1.59	49.7	7.68

*Different superscripts within column indicate significant differences between means ($p<0.05$)

305-day milk yield of cows with no clinical disease was significantly higher than the yield of all other health groups ($p=0.003$) (Table 2.13). Cows with no clinical disease had a mean lactation yield which was 507, 788 and 904 litres greater than cows with subclinical mastitis, reproductive disorders and metabolic disorders, respectively.

Table 2.13: Mean 305-day milk yield (litres) for each health group from fitted lactation curves with percentage variation explained of greater than 20%

Health group	305-day yield (litres)	s.e.m
No clinical disease	10209^{a}	100.6
Subclinical mastitis	9702^{b}	285.9
Reproductive	9421^{b}	223.0
Metabolic	9305^{b}	418.3

*Different superscripts within column indicate significant differences between means ($p<0.05$)

Significant differences in mean peak yield and 305-day yield existed between groups using both methodologies; when data from all fitted lactation curves was analysed and when data analysis performed on a restricted subset of data to exclude curves which had less than 20% variation in milk yield explained by the model fitted lactation curve. Time to peak was significantly different between groups in the full dataset however, it was not significantly different when data from poorly fitted curves was removed.

2.4.3 Fertility

Cows with metabolic disease in the first 30 days of lactation had a significantly ($p<0.01$) greater interval between calving and first observed heat compared to all other health groups (Table 2.14). On average, cows with metabolic disease first observed heat occurred 27 days later in lactation than that of cows with no clinical disease, which equates to just over one reproductive cycle (21 days). No difference was found in the days to first observed heat interval between cows with no clinical disease and those that were diagnosed with subclinical mastitis or reproductive disorders. Consequently, cows with metabolic disease had a significantly increased ($p<0.01$) interval between calving and first service compared to all other groups. On average, cows with metabolic disease were first served 26 days later in lactation than cows with no clinical disease.

Table 2.14: Least square mean days to first observed heat and mean days to first service for each health group

	Days to first observed heat		**Days to first service**	
Health group	**Least square mean (days)**	**s.e.m**	**Least square mean (days)**	**s.e.m**
No clinical disease	63^{b}	3	69^{b}	3.5
Subclinical mastitis	60^{b}	5	72^{b}	5.4
Reproductive	69^{b}	4	74^{b}	4.5
Metabolic	90^{a}	8	95^{a}	7.5

*Different superscripts within column indicate significant differences between means ($p<0.01$)

The mean number of times each cow was served to establish pregnancy was not significantly different between cows of different health groups and only ranged by 0.4 services (Table 2.15). The maximum number of services per cow lactation was highest in cows with no clinical disease and lowest in those which had mastitis in the first 30 days of lactation. The median number of services per conception was highest in cows which had suffered from reproductive disorders, at 3 services per cow lactation, compared to 2 in all other health groups.

Table 2.15: Least square mean number of services per conception and descriptive statistics

Health group	**LSMean (n)**	**s.e.m**	**Maximum (n)**	**Mode (n)**	**Median (n)**
No clinical disease	2.61	0.24	12	1	2
Subclinical mastitis	2.64	0.38	6	1	2
Reproductive	2.67	0.31	8	1	3
Metabolic	2.71	0.51	9	1	2

Mean calving interval (from the calving in the lactation in which disease status was classified until the next calving) was not significantly different between different health groups (Table 2.16). However, minimum calving interval in the metabolic group was 29 days greater than the minimum calving interval of all other groups. Similarly, median calving interval was highest in the metabolic group, at 462 days, which was 62 days greater than that of cows with no clinical disease. Maximum calving interval (662 days) was observed in the group with no clinical disease

Table 2.16: Descriptive statistics for calving interval (days) for each health group

Health group	**LSMean (days)**	**s.e.m**	**Minimum (days)**	**Median (days)**	**Maximum (days)**
No clinical disease	402	8.4	351	400	662
Subclinical mastitis	412	13.3	351	414	537
Reproductive	413	10.9	351	423	607
Metabolic	430	18.4	380	462	640

Mean conception rates at first service ranged from 31 to 45% according to health group, although no significant differences existed between groups (Table 2.17).

Table 2.17: Conception rate at first service (% of cows confirmed in calf to the first service *post*-calving) for each health group

Health group	**Conception to first service rate (%)**
No clinical disease	35
Subclinical mastitis	45
Reproductive	31
Metabolic	43

The 100 day in-calf rate was significantly different between health groups (p=0.049) (Table 2.18). Cows with a reproductive disorder in early lactation had the lowest 100 day in-calf rate (25%) which was substantially lower than that recorded amongst the cows with no clinical disease.

Table 2.18: 100 day in-calf rate (% of cows confirmed in calf at 100 days in milk) for each health group

Health group	**100 day in-calf rate (%)***
No clinical disease	45
Subclinical mastitis	30
Reproductive	25
Metabolic	38

*There are no *post hoc* tests available for use in this analysis, so although a significant difference exists, no superscripts can be added.

2.4.4 Culling

Fertility was the main eventual cull reason for all cows in the study and accounted for 25.4 % of culls. (Figure 2.4). 27.1% of culls did not have any reason specified. “Other” eventual cull reasons included internal bleeding, hernia, unspecified *post*-calving issues and wasting. Accidents included damaged and broken leg, hip or stifle joint and accounted for 7.9% of culls.

The proportion of cows eventually culled for fertility related reasons was significantly different between health groups (p<0.001, Pearson chi-square = 76.58) (Table 2.19). Of cows diagnosed with reproductive disorders in early lactation, 85% were eventually culled due to fertility related reasons compared to less than 20% fertility related culls in all other health groups (Table 2.19). The proportion of cows eventually culled for mastitis was not significantly between health groups and accounted for 10.2 – 18.5% of eventual culls across the health groups.

Figure 2.4: Pie chart showing eventual cull reason as a proportion of all culled animals

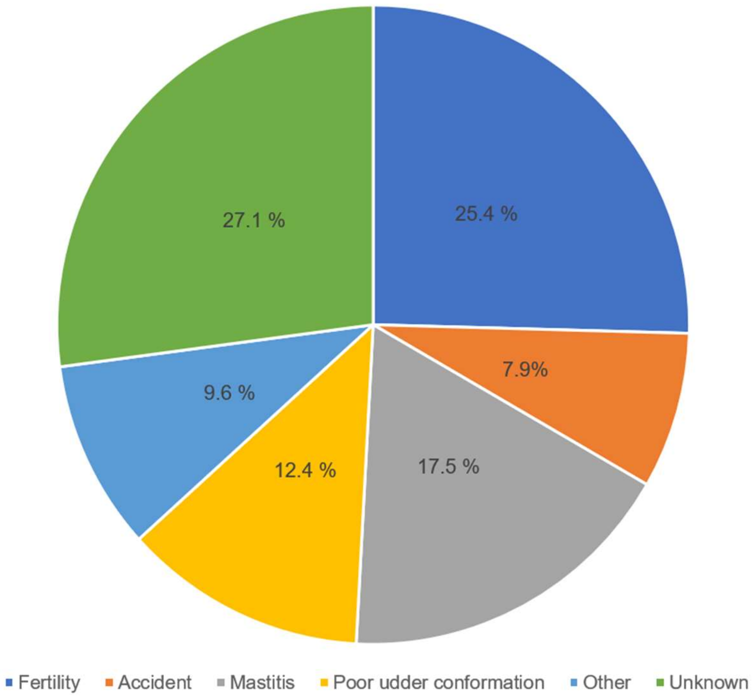


Table 2:19: Cull rates for fertility and mastitis as proportion of total culls for each health group

Health group	Culled for fertility (% of culls)	Culled for mastitis (% of culls)
No clinical disease	19.3	18.5
Subclinical mastitis	18.2	13.6
Reproductive	83.1	10.2
Metabolic	11.1	11.1

Overall cull rates at the end of the study period were significantly different between health groups (p<0.001, Pearson chi-square = 36.51). At the end of 2011, 64.9% of cows that had been classified as having no clinical disease were still alive i.e. 35%

had been culled. Cull rates among the other 3 health groups were between 24 – 36% greater than that seen in the no clinical disease group (Table 2.20).

Table 2:20: Cull rates at end of 2011 (end of study period) for each health group

Health group	**Cull rate at end of 2011 (%)**
No clinical disease	35.1
Subclinical mastitis	71.0
Reproductive	60.0
Metabolic	59.4

2.5 Discussion

The objective of this study was to quantify the effect of early lactation disease on measures of milk yield, fertility and culling. It was hypothesised that cows with disease in the first 30 days of lactation would have reduced productivity compared to cows that remained healthy in the same period. This study has demonstrated that production, as measured by milk yield and fertility, is different between cows of different *post*-calving health status. Significant differences in milk production and fertility exist both between apparently healthy and non-healthy cows and between diseases within the sub-population of non-healthy cows. Furthermore, reasons for culling and the overall cull rate were different between cows of different *post*-calving health status.

(i) Milk yield

Peak milk yield and total 305-day yield were affected by disease status in early lactation; cows with no clinical disease achieved significantly higher peak milk yield and 305-day yields compared to cows with any of the diseases included in this study. Peak milk yield of cows with reproductive disorders was reduced by 1.9 litres compared to cows with no clinical disease and cumulative milk yield losses over lactation totalled 788 litres in cows with reproductive disorders. A very similar result was recently reported by Perez-Baez *et al*. (2021) who found that 305-day yield was reduced by 813.9kg in cows diagnosed with metritis compared to cows without metritis. In a comparable study, milk yield of cows with retained placenta was decreased by 2.6 kg per day in *multiparous* cows through the first 4 milk recordings of the lactation (Dubuc *et al*., 2011). In a study by Machado *et al*. (2020) cows diagnosed with metritis before 8 days in milk produced less milk in the first 2 months of lactation compared to cows that did not develop metritis. However, there was no difference between the milk yields of cows diagnosed with metritis after 8 days in lactation and cows that did not develop metritis. Their finding of reduced milk yield agrees with the current study, although the timing of diagnosis was not analysed in the current study. Encouragingly, effective treatment of metritis has been shown to mitigate some of milk yield losses associated with the disease – Figueiredo *et al*. (2021) found that cows which had a clinical cure by day 10 after diagnosis of metritis had a mean daily milk yield of 40.6kg/day for the first 10 months of lactation whereas cows that were not cured within 10 days of diagnosis had a mean daily milk yield of

37.7kg/day for the same period. Cows which were not affected by metritis had a mean daily milk yield of 42.0kg/day, 1.4kg/day greater than cows with metritis which was cured within 10 days of diagnosis.

Similar to milk yield losses associated with reproductive disorders, the current study also found that cows with metabolic disease had mean peak milk yields 4.2 litres less than cows with no clinical disease and a total 305-day yield which was 904 litres less than apparently healthy cows. Previous studies have reported short-term losses of between 3 and 6 kg/day following an incidence of metabolic disease (Fourichon *et al.*, 1999; Ostergaard and Grohn, 1999). Mean peak milk yield of cows with subclinical mastitis in the first 30 days of lactation was also reduced by 1.9 litres, with cumulative losses totalling 507 litres over 305 days. A very similar result was reported by Fernandes *et al.* (2020) who found that cows diagnosed with subclinical mastitis within a month of calving had a reduction in mean daily milk yield of 1.4 litres for the first 10 months of lactation compared to cows that remained healthy. Relative to clinical mastitis in multiparous cows, cumulative milk losses of 253kg for the first episode of mastitis, 238kg for the second episode and 216kg for the third episode have been reported (Bar *et al.*, 2007). Elucidation of the relationship between mastitis and milk yield is complicated as in addition to the reduction in milk yield typically seen around the time of disease, high milk yield has previously been shown to put cows at increased risk of mastitis and a correlation between mastitis and yield in the previous lactation is probable (Erb *et al.*, 1987; Fleischer *et al.*, 2001). The negative effect of disease on milk yield *post*-disease can often mask the effect of a predisposing high milk yield prior to disease diagnosis (Fleischer *et al.*, 2001). Analysis of the genetic indexes for milk production relative to health group did not reveal any significant differences. However, cows in the subclinical mastitis group did numerically have the highest PTA for milk yield (kg) suggesting that this group of cows may have had potential for higher milk yields which could have put them at greater risk of developing subclinical and clinical mastitis than their lower-yielding cohorts. Loss of milk yield, as a symptom of disease, meant that milk yield was reduced to less than that of cows with no clinical disease, whose PTA for milk yield was 32kg less than that of cows diagnosed with subclinical mastitis.

Previous studies have demonstrated that there is little or no difference between the timing of peak yield in genetically high and low yielding cows (Ferris *et al*., 1985). The current study showed no difference in the timing of peak yields in healthy and sick animals when data from poorly fitting lactation curves were removed from the analysis however, there were significant differences when all data were included. Dealing with these differing results is problematic; including data from the badly fitting curves may result in including erroneous estimates of yield and time to peak yield but removing them is not random and therefore could bias the overall results. In a study by Hostens *et al*. (2012) who used a similar method of modelling lactation curves, time to peak yield amongst cows with retained placenta was delayed.

One of the major drivers for disease-mediated milk yield losses is likely to be a decrease in feed intake amongst sick animals. Bell and Roberts (2007) found that cows with uterine infections had significantly reduced dry matter intakes during the first 100 days of lactation. Reductions in feed intake in the dry period (i.e. prior to disease onset) have also been reported for cows which go on to develop uterine infections *post*-calving (Huzzey *et al*., 2007). This reduction in feed intake could result in less energy being available for milk synthesis and therefore, milk production decreases.

A new and emerging field of research is pointing to an alternative potential reason for disease-related milk yield losses related to the competitive allocation dynamics of glucose for milk production versus immune cell function (Habel & Sundrum, 2020). Glucose is the most essential fuel for both immune cell function and for mammary epithelial cells which synthesise milk, and in early lactation when negative energy balance is inevitable, glucose supply is limited and there is a trade-off between these cell types (Habel & Sundrum, 2020). Kvidera *et al*. (2017) reported that an acutely activated immune system in a Holstein cow uses in excess 1kg of glucose within 12 hours and this causes a redirection of glucose to immune functions and consequently deprioritises milk synthesis. Milk yield was reduced by approximately 80% amongst cows with activated immune systems compared to control cows (Kvidera *et al*., 2017). In cows with acute clinical disease, there is therefore likely to be reduced glucose available for milk synthesis resulting in a reduction in daily milk yields.

(ii) Fertility

In the current study, resumption of normal reproductive activity (i.e. oestrus) was delayed in cows which had suffered from metabolic disease in the first 30 days of lactation and did not recommence until 90 days after calving. Consequently, the interval from calving to first service was extended in these cows. This result agrees with the work of Fourichon *et al*. (2000) who reported an 8-day increase in the calving to first service interval in cows diagnosed with subclinical ketosis. Such a delay in the resumption of reproductive activity suggests that the cow is prioritising her present physiological needs over the "need", created by modern farming practices, to become pregnant as soon as possible after calving (Friggens *et al*, 2003). Reproductive activity may be delayed until the cow is in an optimum physiological state for re-breeding. Fertility, defined as the ability of an animal to conceive and maintain pregnancy if inseminated at the appropriate time in relation to ovulation, is one of the key pillars of dairy production (Darwash *et. al*, 1997; LeBlanc, 2008). Reproductive efficiency, which ensures continued milk production following the birth of offspring, is one of the key determinants of farm profitability (Meadows *et al*., 2005). Sub-optimal reproductive performance has a negative effect on the quantity of milk produced per cow, thereby reducing outputs from dairy production system while simultaneously increasing inputs in the form of veterinary costs (Lawson *et al*., 2004). Long term or repeated reproductive failure leads to increased culling rates and thus increases replacement rates and costs.

In the current study there was no extension of the intervals from calving to either first heat or first service in cows which had reproductive disease in relation to cows which were apparently healthy in early lactation. Similarly, calving to both first heat and first service were not extended in cows diagnosed with a metabolic disease. Additionally, conception rate at first service was not significantly different between health groups and was equal or greater to the industry target of 35% in all groups apart from cows diagnosed with reproductive disorders. These results contrast with much of the published work in this field. In their review of 2000, Fourichon *et al*. summarised the literature investigating the link between disease and fertility and found that clinical ketosis and retained placenta were associated with a 4 – 10% reduction in conception rate at first service and metritis was associated with a 20% lower conception rate at first service. In a retrospective study of data from 7500 lactating cows, Carvalho et.

al. (2019) looked at *postpartum* disease holistically and reported a 19% reduction in pregnancy rate in cows diagnosed with at least one clinical disease in the first 21 days of lactation. In a large-scale observational study of over 11,000 cows, reproductive disease had a negative effect on the odds of a cow becoming pregnant at first service. However, there was no effect of subclinical ketosis, mastitis, displaced abomasum or pneumonia on conception to first service rate (Pindeo *et al.*, 2020).

The 100 day in-calf rate was however significantly affected by health group; cows with reproductive disease had a 100 day in-calf rate of 30% compared to 45% amongst cows with no clinical disease. That the 100 day in-calf rate was lower than the conception to first service rate is reflective of the fact that a proportion of cows did not receive their first service until after 100 days *post*-calving. This is particularly seen in the metabolic group where the mean days to first service was 95. The finding of reduced 100 day in-calf rate amongst the reproductive health group accords with the results of Ernstberger *et al*. (2019) who reported that, at 150 days in milk, 48% of cows which had suffered from metritis earlier in lactation were pregnant compared to 68% of cows which did not have metritis.

(iii) Culling

As cows were only maintained in the long-term genetics x environment study for 3 lactations the culling rate was low; of the cows in the current analysis only 11.3% were culled or died before completion of lactation 3. The average age of death of cows in the current study was 6.3 years. This made analysis of culling data problematic due to the low numbers of cows culled within the first 3 lactations and thus necessitated the use of data from out with the official study period to examine eventual cull reasons.

The main eventual cull reasons amongst all cows included in the study were similar to those reported in the literature. In a survey of conventional Swedish Holstein herds, 22.5% of culls were found to be due to udder health and 24.8% were due to poor fertility (Ahlman *et al.*, 2011). Data from the national milk recording scheme in the United States reported that 18.9% of culls were due to reproduction and 12.1% were due to mastitis (Hadley *et al.*, 2006). In 2018, the 3 most common reasons for culling in Canadian dairy herds were reproductive problems (17%), mastitis (11%) and foot and leg problems (7%) (Stojkov *et al.*, 2020).

Eighty five percent of cows which were diagnosed with reproductive disorders within 30 days of calving in lactation 2 or 3 were eventually culled due for fertility related reasons. This was considerably higher than the proportion of cows culled for fertility in any of the other health groups and is indicative of the fact that these cows are likely to have had ongoing poor fertility which predisposed them to a higher risk of culling. Furthermore, overall cull rate at the end of the study period was 24.9% higher in cows with reproductive disease than in cows with no clinical disease. This result is consistent with much of the literature which reports that risk of culling is significantly higher for cows with poor fertility (Ansari-Lari *et al.*, 2012). Cows with diagnoses of follicular ovarian cysts (a reproductive associated problem) were found to have 1.5 times increase in the rate of culling compared to healthy cows (Erb *et al.*, 1985).

High somatic cell count and treatment of mastitis have been associated with an increased risk for culling (Gussmann *et al.*, 2019). There was no significant difference in the proportion of cows culled for mastitis between health groups in the current study. However, the overall cull rate at the end of the study period was highest (71%) amongst cows which had had subclinical mastitis in early lactation. Beaudeau *et al.* (1995) reported that high somatic cell count was associated with a higher risk of being culled; cows with a monthly cell count record of greater than 800,000 cells/ml had a 1.7 times higher risk of being culled than cows with somatic cell counts less than 300,000 cells/ml. In order to more fully understand the effect of mastitis on culling in the current study it may have been helpful to look at the nature of the high cell count i.e. was it a “one off” occurrence or was it elevated for an extended period of time

In the current study, cows with metabolic disease did have an increased cull rate over the study period of 24.3% compared to cows with no clinical disease, but the low numbers of cows in this sub-population mean that each cow had a very large influence on the cull rate. The association between metabolic disease and culling risk has been comprehensively reported by Probo *et al.* (2018) who found that culling risk at 120 days in milk was 13% for healthy cows compared to 25% for cows with one metabolic disease and 33% for cows with complicated metabolic disease.

(iv) Challenges and limitations of the study

All disease in this study was naturally occurring and therefore the number of cases was not balanced across parities and production systems. The benefit of analysing data from cattle with naturally occurring disease is that the disease follows its natural trajectory, much like it would in a normal dairy herd setting. However, this fact, combined with the very good level of herd management at the SRUC Dairy Centre gave rise to a very low incidence of disease and meant that sample size was small. This is reflected in the small size of the metabolic group used throughout this study (n=17) and the inflated standard error of means seen for each parameter investigated for this group. Hackshaw *et al*. (2008) succinctly summarised the effects of study size on conclusions; a large study generates small standard errors with a narrow 95% confidence interval meaning there is a precise estimate of the effect and that firm conclusions can be made. In contrast, a small study results in a large standard error with a wide 95% confidence interval and means estimates of the effect may be imprecise and therefore no firm conclusions can be made. For example, a study including 20 subjects is likely to be too small for most investigations. In the context of the current study, if the proportion of cows with poor conception rates among a particular group of 20 cows is 25%, the associated 95% confidence interval is 9 – 49 which means that the true prevalence in these subjects is generally anywhere below a low or high value which is not a useful result (Hackshaw, 2008).

A key strength of the data source used in the current study is that the management practices, including diet formulation and breeding policies, are very well defined and have been variously described in the literature. This means that the effects of diet, genetic line and the interaction between these can be accurately accounted for in any statistical tests which would not be the case if data from many herds was collected in a survey-type study. Conversely, a weakness of the current study is that it was performed on one herd which means there is limited replication of the results at herd-level i.e. how similar are the results found in one herd to those which would be found in another herd? This weakness is somewhat mitigated by the existence of the 4 sub-herds (high forage control, high forage select, low forage control & low forage select) within the herd as most commercial dairy herds in the UK will be similar to one of these 4 systems. A key challenge in the long-term genetics x environment study is the maintenance of the 2 genetic lines – control and select. The “control” group in this study is rolling in nature; that is to say, it has changed over time since the beginning of the long-term study in the 1970s. In the 1970s the control line represented the average genetic merit of dairy cows in the UK at that time however, it now represents the average genetic merit of dairy cows in the UK today. A larger difference between the control and select lines, which may improve statistical power, would exist if the control line had been static i.e. remained as it was at the start of the long-term study. Nonetheless, the average difference in milk yield was large at over 2000kg/lactation and in the many published studies generated from this long-term study, genetic line tends to be one of the largest effects e.g. Veerkamp *et al*. (1994), Pryce *et al*. (2001), Ross *et al*. (2014) and Randall *et al*. (2015). Maintaining the control line as a static control group would be biologically interesting but would not be commercially viable or relevant to modern genotypes.

2.6 Conclusion

The current study has demonstrated that productivity of dairy cattle, as measured by milk production, fertility and culling rates, is different among cows of different disease status *post*-calving. Quantifying the effect of disease on productivity demonstrates that alongside the important welfare implications of disease, there is an economic justification for improving health. In the case of short-term losses in milk yield around the time of disease, a reduction in saleable milk has obvious financial repercussions. Due to the clear impact of disease on productivity, economics and welfare, reduction of disease incidence and the early detection of disease to facilitate successful treatment is essential.

Chapter Three

Use of Data from the Dry Period to Identify Candidate Indicators of Production Disease in the Transition Period

3.1 Introduction

Prompt disease detection is fundamental to good herd health management. Timely identification of sick animals enables appropriate treatments to be implemented, reducing disease severity and preventing the development of chronic conditions. Consequently, prompt disease detection offers the opportunity to minimise the economic losses associated with disease and improve dairy herd profitability (Probo *et al*., 2018). Timely detection of disease, though a vital component of health management, is challenging in the modern dairy herd, the average size of which continues to increase (AHDB, 2019). Fewer staff manage an increasing number of cattle, and accordingly time available for individual animal observation and the identification of sick animals is reduced (LeBlanc, 2010). Thus, there is a need for the development of automated precision management tools which facilitate individual cow monitoring.

In dairy production, automated tools and precision farming techniques are becoming a vital component of management systems. Such tools and techniques are especially important in the transition period, because of the physiological shifts that characterise this period. The transition period is defined as the period which extends from three weeks before parturition to three weeks *postpartum* (Nordlund & Cook, 2004). During this time, late term foetal growth, parturition and the initiation of lactation are accompanied by significant endocrine changes which exceed those occurring at any other stage in the dairy cows' production cycle (Grummer *et al*., 2004). Further, cows are subject to significant changes in management throughout the transition period which include dietary alterations and changes in housing conditions.

Immediately after calving, and despite feed intake being at its lowest point in the lactation-gestation cycle, cows experience a significant increase in nutrient requirements to facilitate the competing processes of milk production, resumption of ovarian activity and immune system activation (Grummer *et al*., 2004; Lucy *et al*., 2014). This combination inevitably results in a state of negative energy balance (NEB) (Frigo *et al*., 2010). Throughout the lactation-gestation cycle the cow alternately accumulates and depletes lipid reserves. Lipid reserves are accumulated throughout pregnancy in anticipation of lactation, before being mobilised *post*-calving to reach optimal condition for re-breeding (Friggens, 2003). However, management mediated disruptions to this cycle which extend the period of NEB or cause a more severe NEB have been shown to be linked with increased levels of metabolic and production disease in early lactation (Ingvartsen, 2003; Cardoso *et al*., 2020). Excessive tissue mobilisation caused by an extended or severe period of NEB is a major driver of inflammation, further increasing the cow's energy requirements which can cause a self-perpetuating process of inflammation-driven tissue mobilisation resulting in severe oxidative stress and immune suppression (Abuello *et al*., 2015; Contreras *et al*., 2017).

Consequently, in high yielding cows approximately 75% of diseases occur within 30 days of calving (LeBlanc, 2010). The effects of such diseases have direct consequences on the productivity and duration of the ensuing lactation (Vergara *et al*., 2014). Therefore, prompt disease detection in the transition period would be of great value as swift remedial action would allow the establishment of a healthy and profitable lactation and the mitigation of poor welfare and diseases. Lukas *et al*. (2009) suggested that alerts to highlight individual animals requiring special attention or those at risk of developing disease would be a useful management tool for producers during this high-risk period.

Although considerable progress has been made in the use of technology in general dairy production, there is still a need for specific tools to be developed for the detection of metabolic and production diseases. In most instances, it is still unclear as to which parameters of routine herd data are the most appropriate indicators of disease, particularly in the dry and transition periods (Rutten *et al*., 2013; de Vries *et al*., 2011). Most studies and hence interventions have focused on monitoring systems for use in lactation e.g. using milk-based parameters to detect mastitis (Homer *et al*., 2013) and activity monitors for the detection of oestrus (Jonsson *et al*., 2011), or involve the collection of samples which are not readily available on commercial dairy farms e.g.

blood samples to predict disease risk through immunological and chemical means (Amadori *et al*., 2015), and urine samples to screen for individual cow susceptibility to subclinical mastitis (Zwierzchowski *et al*., 2020).

Although many risk factors for individual diseases have been identified, sensor systems for metabolic diseases, which are closely associated with the dry and transition periods, have not been extensively researched (Rutten *et al*., 2013). In contrast, parameters associated with an increased risk of clinical mastitis (e.g. somatic cell count) have long been used to inform management decisions and thus reduce the risk of future disease (Bradley and Green, 2000; Pantoja *et al*., 2009).

The identification of production and physiological measures in late lactation and the dry period which are strongly related to disease risk in the early stage of the following lactation and can be practically measured on commercial dairy farms is crucial to the further development of automated means of disease detection (Huybrechts *et al*, 2014; Lukas *et al*, 2014). Extracting features from data that are already recorded in commercial production systems for use as candidate disease indicators offers a cost-effective and innovative method of furthering the development of disease detection models. To develop predictors of transition disease, it is first necessary to identify candidate performance and physiological measures from the end of lactation and dry period and examine them according to health outcomes in early lactation. By necessity, this analysis must be performed retrospectively with cows of known disease status *post*-calving.

3.2 Hypothesis & Objectives

The hypothesis of this study was that cows which develop disease in the first 30 days of lactation would exhibit different changes in their physiology and productivity at the end of the preceding lactation and throughout the dry period, compared to cows that remained healthy after calving. In addition, it was hypothesised that there would be differences in physiology and productivity between cows that developed different diseases in the first 30 days of lactation.

Therefore, the objective of this study was to identify candidate measures of physiology and productivity from the late lactation and dry periods which can be used to distinguish between healthy and non-healthy cows. This was performed with a view to the future inclusion of such candidate measures in disease detection models.

3.3 Materials & Methods

3.3.1 Data source

Data were obtained for this study from the herd of Holstein-Friesian cattle at Scotland's Rural College's (SRUC) Dairy Research and Innovation Centre, Crichton Royal Farm, Dumfries. Data used were from a period of eight years, from November 2003 to September 2011, when cattle were on a long-term 2x2 factorial experiment which investigated the interaction between genotype and environment (Pryce *et al.*, 2001). For full details of herd management and data collection see Chapter 2 Materials & Methods.

3.3.2 Data Handling

Cow-lactations were used as the experimental unit throughout the analysis. The total number of cow-lactations eligible for use in this study was 482, from 399 individual cows moving between lactations 1 and 2, and lactations 2 and 3. Distribution of these cow-lactations across production system and parity are presented in Table 2.9. As discussed in Chapter 2 Materials & Methods, lactation 1 data was excluded from this analysis due to the differences in physiology and management between maiden heifers and dry cows.

3.3.2.1 Classification of cow-lactations

All cow-lactations were assigned to 1 of 4 groups based on disease incidence in the first 30 days of the on-going lactation. These groups were no clinical disease (NCD), reproductive (REP), mastitis (MAST) and metabolic (MET). To qualify for inclusion in 1 of the 4 health categories, cow-lactations were subject to the same criteria presented in Table 2.7.

Cows diagnosed with more than one disease were assigned to the health group of the most severe disease (Goff, 2006). The number of cow-lactations with multiple disease diagnoses in the first 30 days of lactation accounted for 0.2% of the cow-lactation records used in this study. Three cow-lactations had a record of subclinical mastitis and a reproductive disorder (classified as reproductive disorder), 4 had a record mastitis and a metabolic disorder (classified as metabolic) and 5 had a record of reproductive and metabolic disorder (classified as metabolic).

3.3.4 Candidate indicator traits

A total of 14 candidate indicators were identified as detailed.

1. Body weight at dry-off (Ind1)
2. Body weight at calving (Ind2)
3. Body condition score at dry-off (Ind3)
4. Body condition score at calving (Ind4)
5. Body energy content at dry-off (Ind5)
6. Body energy content at calving (Ind6)
7. Body weight change (Ind7)
8. Body condition score change (Ind8)
9. Body energy content change (Ind9)
10. Body weight slope (Ind10)
11. Body condition score slope (Ind11)
12. Body energy content slope (Ind12)
13. Milk yield at dry-off (Ind13)
14. Milk yield at dry-off: body energy at calving ratio (MBER) (Ind14)

Knowledge gained whilst conducting the literature review pointed to the strong relationship between energy status (as measured by body weight and body condition score) and future health status, therefore it was decided that this should be the key focus of research.

Body weight at dry-off (1), body weight at calving (2), body condition score at dry-off (3) and body condition score at calving (4) were extracted from the data generated by the walkover weigh scales and farm records. Body energy content at dry-off (5) and body energy content at calving (6) were calculated using the method given by Banos *et al*. (2006) utilising the US National Research Council (NRC) equations. Weekly liveweight and BCS records from the dry period were used to calculate lipid and protein weights as described.

$$\text{Body lipid weight (kg)} = (0.037683 \times \text{BCS}) \times (\text{empty body weight})$$

$$\text{Body protein weight (kg)} = [0.200886 \times (0.0066762 \times \text{BCS})] \times (\text{empty body weight})$$

These equations were designed for use with a 1 – 9 BCS scale, therefore for calculation of body energy content, the raw BCS data was subject to a conversion prior to the calculation of body lipid and protein weights using the following equation.

$$BCS9 = (BCS5 - 1) \times 2 + 1.$$

Empty body weight (kg) was calculated as a function of live weight (kg) and day of gestation (dp) as follows.

$$\text{Empty body weight} = ((\text{live weight}) \times 0.96) - (0.7312 \times \exp(0.02 \times dp - 0.0000143 \times dp \times dp)) \times 0.851$$

Estimated body lipid and protein weights were then combined to predict energy content (in MJ) using the following formula (NRC, 2001)

$$BEC\ (MJ) = [(9.4 \times (\text{Body lipid weight})] + [5.7 \times (\text{Body protein weight})] \times 4.1868$$

Changes in BW, BCS and BEC over the dry period were calculated as.

BW change = BW at drying off – BW at calving (Ind7)
BCS change = BCS at drying off – BCS at calving (Ind8)
BEC change = BEC at drying off – BEC at calving (Ind9)

An intercept and slope for body weight, body condition score and body energy content were calculated from the weekly measurements for the first 15 days of the dry period, irrespective of dry period length. Slopes of change in body weight (Ind10), body condition score (Ind11) and body energy content (Ind12) were calculated for the first 15 days of the dry period to determine the effects of this critical period on future health status. Changes and slopes in BW, BCS and BEC were calculated to capture the rate of change in the respective traits throughout the dry period, as previous work had suggested that relative changes in energy status is more important in determining health outcomes when compared to absolute values of body weight or body condition. Milk yield at dry-off (Ind13) was determined on the last day of lactation on which the cow was milked three times. A novel candidate indicator of milk yield: body energy ratio (MBER) (Ind14) was calculated from milk yield and BEC data as daily energy corrected milk (ECM) (litres) per one hundred MJ of cows' daily body energy content at dry off. This was calculated as a basic or proxy measure to determine whether the cow was prioritising milk yield or body condition at the end of lactation, as ample evidence exists that genetic drives for life functions other than milk imply that nutrient partitioning will change through lactation (Friggens and Newbold, 2007). It was hypothesised that cows "prioritising" milk yield and those "prioritising" body condition score may have differences in disease incidence *post*-calving. Daily milk yield was converted to ECM, using the method by Sjaunja *et al*. (1990). The equation used was ECM (kg) = 0.25M + 12.2F + 7.7P, where M is milk yield (kg), F is fat content (g kg^{-1}) and P is protein content (g kg^{-1}).

3.5 Data Analysis

Descriptive statistics were used to summarise the data and study the initial profiles and trends. Analysis of variance (ANOVA) employing a generalised linear model (GLM) was used to determine effects for all the candidate indicator traits. All analyses were conducted using the GLM procedure of SAS 9.3 (SAS Institute Inc., 2010). The model used was:

$$y_{ijklmno} = \mu + S_i + H_k + D_l + P_m + A_n + C_o + \varepsilon_{ijklmno}$$

where y was the candidate indicator trait (each of the 14 candidate indicators previously described); μ is the overall mean; S_i is the fixed effect of dairy production system (LFC, LFS, HFC, HFS); H_k was the fixed effect of health group (1,2,3,4,5); D_l was the fixed effect of dry period length; P_m was the fixed effect of parity (2 or 3); A_n was the fixed effect of calendar year; C_o was the random effect of individual cow; $\varepsilon_{ijklmno}$ was the random error term. Calf birth weight and the presence of twinning were both found to be highly correlated to dry period length and were therefore not included as covariates. Significant differences between variables were determined by pairwise comparisons using the Tukey method.

3.4 Results

3.4.1 Body Energy Content

3.4.1.1 Analysis of Variance

From the analysis of variance, it was seen that production system significantly affected all four derivatives of BEC ($p< 0.05$). Parity had a significant effect on body energy content at drying off (doBEC), body energy content at calving (calvBEC) and the difference between body energy content at drying and calving (diffBEC) ($p< 0.01$) but had no effect on slope15BEC. Health group significantly affected with slope15BEC ($p= 0.02$) and diffBEC ($p< 0.001$). Calendar year had significant effect ($p< 0.001$) on doBEC and calvBEC. Least square means and standard deviations for each factor and level are presented in Table 3.1.

BEC at drying off was significantly higher ($p < 0.001$) in Low Forage Control (3443 MJ) and Low Forage Select (3340 MJ) cows than in both High Forage Control (2948 MJ) and High Forage Select (2808 MJ) cows. At the time of calving, BEC was significantly different between Low Forage Select and High Forage Select cows ($p= 0.02$). Both Low Forage Control and Low Forage Select cows had a negative slope of change in BEC in the first 15 days of the dry period. The slope of change experienced by Low Forage Select cows (-13.73 MJ/day) was significantly different than that of High Forage Control cows and High Forage Select cows ($p= 0.002$). Both High Forage Control and High Forage Select cows had positive slopes of change, High Forage Control cows had a slope of 9.97 MJ/day and High Forage Select cows had a slope of 6.25 MJ/day. The difference in body energy content from drying off to calving was significantly greater ($p< 0.001$) in Low Forage Control and Low Forage Select than in High Forage Select and High Forage Control cows.

Body energy content was significantly higher in cows progressing to lactation 3 than in cows progressing to lactation 2 at both drying and calving ($p< 0.001$). No difference existed in the rate of change in the first 15 days of the dry period between the two parities. However, the overall difference in body energy content across the whole dry period was significantly greater in cows progressing to lactation 3 ($p= 0.0035$).

Absolute measures of body energy content at both drying off and calving were not significantly different between cows which had no clinical disease and those that developed disease, or between cows that developed different diseases. The slope of change in body energy content experienced in the first 15 days of the dry period was -18.26 MJ/day for cows that went on to develop *post*-calving reproductive disorders. This was a significantly different rate (p= 0.02) from that experienced by cows which had no clinical disease (0.63 MJ/day). The difference in body energy content between the start and the end of the dry period was significantly greater (p= 0.001) in cows that went on to develop reproductive disorders than in cows with no clinical disease, and in those that developed mastitis in the first 30 days after calving.

Body energy content at drying and calving were both numerically highest in the year 2004; these measures were significantly higher than the same measures in most of the other calendar years (p< 0.001).

Table 3.1: LSMeans with standard errors for body energy content at calving, drying off, body energy content slope and difference between calving and drying energy contents by feeding and genetic groups, parity, health group and year.

Factor	**Level**	**n**	**Body Energy Content (MJ)**			
			Drying off (doBEC)	**Calving (calvBEC)**	**Slope (1st 15 days) (slope15BEC)**	**Difference (diffBEC)**
			LSMean ± SEM (MJ)	**LSMean ± SEM (MJ)**	**LSMean ± SEM (MJ/day)**	**LSMean ± SEM (MJ)**
System	Low Forage Control	138	3443^{a} ±133.1	2818 ^{ab} ± 103.9	-7.44^{ac} ± 7.28	-612^{a} ± 106.7
	Low Forage Select	106	3340^{a} ± 140.5	2852 ^{a} ± 109.1	-13.73^{a} ± 7.58	-493^{a} ± 111.5
	High Forage Control	97	2948^{b} ± 135.7	2673 ^{ab} ± 106.3	9.97^{b} ± 7.38	-222^{b} ± 108.4
	High Forage Select	119	2808^{b} ± 138.3	2602^{b} ± 107.9	6.25 ^{bc} ± 7.50	-149^{b} ± 110.2
Parity	2	265	2929^{b} ±118.6	2586 ^{b} ± 93.4	-3.51 ± 6.50	-311^{b} ± 98.9
	3	195	3353^{a} ± 119.6	2887 ^{a} ± 97.7	1.03 ± 6.57	-427^{a} ± 98.9
Health Group	No clinical disease	339	3059 ± 103.1	2817 ± 79.9	0.63^{a} ± 5.11	-235^{a} ± 74.7
	Subclinical mastitis	69	3058 ± 156.6	2821 ± 125.3	3.00^{ab} ± 9.60	-222^{a} ± 106.6
	Reproductive	37	3278 ±133.2	2735 ± 105.3	-18.26^{b} ± 7.44	-596^{b} ± 101.1
	Metabolic	15	3144 ± 220.7	2573 ± 179.1	9.66^{ab} ± 13.95	-422^{ab} ± 171.3
Year	2003	9	2858^{a} ± 260.3	2528 ^{a}± 206.3	-9.55 ± 17.28	-354 ± 197.8
	2004	51	3711^{b} ± 122.4	3261^{b} ± 97.5	-9.85 ± 8.52	-385 ± 92.7
	2005	77	3303^{a} ± 105.1	2640^{a} ± 83.7	-3.31 ± 6.97	-601 ± 79.6
	2006	63	3066^{a} ± 109.5	2637^{a} ± 87.2	4.05 ± 7.38	-401 ± 83.9
	2007	59	3032^{a} ± 119.9	2611^{a} ± 89.4	-1.09 ± 7.43	-445 ± 85.5
	2008	66	3207^{a} ± 133.4	2912^{a} ± 88.5	-3.59 ± 7.95	-342 ±89.2
	2009	73	3038^{a} ± 105.3	2741^{a} ± 85.2	-11.53 ± 6.93	-288 ± 81.7
	2010	61	3106 ^{a}± 114.1	2590^{a} ± 93.7	10.97 ± 7.64	-415 ±88.9
	2011	1	2891 ^{ab}± 811.5	2706^{ab} ± 628.6	12.83 ± 36.47	-85 ± 586.6

*Different superscripts within factor denote statistical significance at $p < 0.05$

3.4.2 Body condition score

LSMeans and standard errors for body condition score are outlined in Table 3.2. Production system significantly affected doBCS, calvBCS, slope15BCS and diffBCS (p< 0.01). Of the traits examined, parity only significantly affected doBCS (p= 0.01) while disease status only significantly affected diffBCS (p< 0.001). Year of production significantly affected doBCS and calving calvBCS (p< 0.01).

At dry off, High Forage Select cows had a body condition score of 2.19 which was significantly lower (p< 0.001) than cows from all other systems, and Low Forage Control cows had a significantly higher body condition score (2.53) than High Forage Control cows (2.34). At calving, High Forage Select cows had the lowest body condition score (2.09), which was significantly lower than Low Forage Control cows that had an average condition score of 2.26. During the first 15 days of the dry period, the slope of change in body condition score was significantly different (p< 0.01) between Low Forage Select cows, that had a slope of -0.00068 body condition score units (CSU)/day, and High Forage Control cows that had a slope of 0.00955 CSU/day. The difference in body condition score between dry off and calving was significantly different between cows of different production systems (p< 0.001). Low Forage Control cows lost more than twice the amount of BCS compared to High Forage Control and High Forage Select systems.

Drying off body condition score was significantly higher in cows moving to lactation 3 than those moving to lactation 2 (p< 0.01). No other significant differences existed between parity 2 and parity 3 cows for the candidate indicators. The slope of change in body condition score across the first 15 days of the dry period was significantly affected by system (p< 0.01). Low Forage Select cows had a negative slope (-0.00068 CSU/day), which was significantly different to the slope experienced by High Forage Control cows.

Table 3.2: LSMeans with standard errors for body condition score at calving, at drying off, the body energy content slope and the difference between calving and drying by feeding and genetic groups, parity, health group and year

Factor	Level	n	Body Condition Score (CSU)			
			Drying (doBCS)	Calving (calvBCS)	Slope (slope15BCS)	Difference (diffBCS)
			LSMean ± SEM	LSMean ± SEM	LSMean ± SEM	LSMean ± SEM
System	Low Forage Control	138	2.53^{a} ± 0.058	2.26^{a} ± 0.046	0.0022^{ab} ± 0.0039	-0.28^{a} ± 0.047
	Low Forage Select	106	2.44^{ab} ± 0.061	2.19^{ab} ± 0.048	-0.0006^{a} ± 0.0041	-0.26^{a} ± 0.049
	High Forage Control	97	2.34 ± 0.059	2.22^{ab} ± 0.047	0.0095^{b} ± 0.0040	-0.11^{b} ± 0.048
	High Forage Select	119	2.19^{c} ± 0.060	2.09^{b}± 0.048	0.0083^{ab} ±0.0041	-0.09^{c} ± 0.048
Parity	2	265	2.34^{b} ± 0.057	2.17 ± 0.042	0.0036 ±0.0007	-0.17 ± 0.043
	3	195	2.42^{a} ± 0.058	2.21 ± 0.042	0.0061 ± 0.0007	-0.20 ± 0.043
Health Group	No clinical disease	339	2.36 ± 0.045	2.24 ± 0.036	0.0039 ± 0.0006	-0.11^{a} ± 0.036
	Subclinical mastitis	69	2.35 ± 0.069	2.22 ± 0.056	0.0030 ± 0.0008	-0.13^{ab} ± 0.058
	Reproductive	37	2.46 ± 0.058	2.20 ± 0.047	-0.0019 ± 0.0008	-0.27^{b} ± 0.048
	Metabolic	15	2.34 ± 0.099	2.10 ± 0.079	0.0144 ± 0.0013	-0.24^{ab} ± 0.082
Year	2003	9	2.27^{ab} ± 2.09	2.09^{abcd} ± 0.094	0.0367 ± 0.0088	-0.193 ± 0.098
	2004	51	2.62^{b} ± 0.059	2.39^{bcd} ± 0.044	-0.0039 ± 0.0041	-0.219 ± 0.049
	2005	77	2.44^{ab} ± 0.054	2.13^{cd} ± 0.037	0.0020 ± 0.0035	-0.292 ± 0.045
	2006	63	2.38^{a} ± 0.055	2.14^{acd} ±0.039	0.0015 ± 0.0036	-0.248 ± 0.047
	2007	59	2.35^{a} ± 0.057	2.11^{d} ± 0.046	0.0017 ± 0.0036	-0.241 ± 0.048
	2008	66	2.43^{ab}± 0.054	2.28^{abd} ± 0.039	0.0035 ± 0.0036	-0.145 ± 0.045
	2009	73	2.36^{a} ± 0.053	2.24^{acd} ± 0.038	0.0022 ± 0.0035	-0.146 ± 0.045
	2010	61	2.39^{a}± 0.055	2.17^{acd}±0 0.041	0.0055 ± 0.0038	-0.214 ± 0.046
	2011	1	2.15^{ab}± 0.357	2.14^{abcd} ± 0.284	0.0272 ± 0.0187	-0.017 ± 0.290

*Different superscripts within factor denote statistical significance at $p < 0.05$

The difference in body condition score across the dry period was significantly higher ($p < 0.001$) in cows which went on to develop metritis in the first 30 days of lactation than in cows which had no clinical disease in the same period. Those that went on to receive a metritis diagnosis lost on average 0.27 CSU across the dry period whereas those that did not develop clinical disease lost on average 0.11 CSU. In years 2006, 2007, 2009 and 2010, body condition score at dry-off was significantly lower than dry-off condition in the year 2004.

3.4.3 Body Weight

Production system had a significant effect ($p < 0.001$) on all 4 traits derived from body weight. Parity had a significant effect on doLW, calvLW and slope15LW ($p < 0.01$). Health group had a significant effect ($p = 0.0015$) on diffBW while year of production had significant effect ($p < 0.01$) on body weight at dry off, body weight at calving, and dry period BW slope. The size of the fixed effects on body weight are presented in Table 3.3. Control cows in the High Forage system were significantly lighter ($p < 0.001$) at dry off than cows from all other groups: on average they weighed 646kg on the day of dry off. There was no difference in body weight at dry off between Low Forage Control, Low Forage Select and High Forage Select cows. At calving, Low Forage Select cows were numerically the heaviest, weighing on average 652 kg; however, this was not significantly different from High Forage Select cows that weighed 639 kg. High Forage Control cows were significantly lighter ($p < 0.001$) than both Low Forage Select and High Forage Select cows. High Forage Control cows had a positive slope of change in the first 15 days of the dry period of 1.38 kg/day. This was significantly different ($p < 0.001$) from the negative slope of -1.13 kg/day of Low Forage Control cows. Over the whole dry period, Low Forage Control cows lost significantly more bodyweight (57.5kg) than either High Forage Control or High Forage Select cows that lost 35.1 kg and 27.7kg, respectively. Cows transitioning to lactation 3 had higher body weight ($p < 0.001$) than those transitioning to lactation 2 at both drying and calving. Slope of change in body weight in the first 15 days of the dry period was significantly different ($p = 0.01$) between cows of different lactations. Cows moving to lactation 2 lost on average 5.8 kg/day, whilst cows moving to lactation 3 gained 0.82 kg/day during this 15-day period.

Table 3.3: LSMeans and standard errors for body weight at calving, at drying, the body energy content slope and the difference between calving and drying by feeding and genetic groups, parity, health group and year

Factor	Level	n	Body Weight (kg)			
			Drying (doLW)	Calving (calvLW)	Slope (1st 15 days) (slope15LW)	Difference (diffLW)
			LSMean ± SEM (kg)	LSMean ± SEM (kg)	LSMean ± SEM (kg/day)	LSMean ± SEM (kg)
System	Low Forage Control	138	686^{a} ± 11.1	626^{ac} ± 10.3	-1.13^{a} ± 0.85	-57.5^{a} ± 7.85
	Low Forage Select	106	695^{a} ± 11.7	652^{b} ± 10.8	-0.38^{ab} ± 0.89	-41.7^{ab} ± 8.21
	High Forage Control	97	646^{b} ± 11.2	610^{c} ±10.5	1.38^{b} ±0.86	-35.1^{b} ± 7.95
	High Forage Select	119	671^{a} ± 11.6	639^{ab} ± 10.8	0.59^{ab} ± 0.87	-27.7^{b} ± 8.09
Parity	2	265	645^{b} ± 9.8	605^{b} ± 9.3	-0.58^{b} ±0.76	-36.9 ± 7.18
	3	195	704^{a} ± 9.9	659^{a} ± 9.4	0.82^{a} ± 0.77	-44.0 ± 7.24
Health Group	No clinical disease	339	667 ±7.9	632 ± 7.5	0.59 ± 0.59	-35.6^{a} ± 6.06
	Subclinical mastitis	69	666 ± 10.3	642 ± 10.3	0.66 ± 1.11	-20.1^{a} ± 9.65^{a}
	Reproductive	37	674 ± 9.9	624 ± 9.7	-1.18 ± 0.87	-55.2^{b} ± 8.08^{b}
	Metabolic	15	693 ±15.4	628 ± 16.2	0.40 ± 1.64	-51.1^{ab} ± 13.99^{ab}
Year	2003	9	658^{ab} ±18.1	618^{abc} ± 18.7	-1.23^{abc} ± 2.04	-43.4 ±16.21
	2004	51	706^{b} ± 9.6	673^{bc} ± 9.5	2.30^{bc} ± 0.94	-28.5 ± 7.52
	2005	77	688^{b} ± 8.9	635^{a} ± 8.6	-0.25^{abc} ±0.80	-50.6 ± 6.46
	2006	63	662^{a} ± 8.9	629^{ac} ± 8.8	1.65^{ab} ±0.87	-29.9 ± 6.87
	2007	59	661^{a} ± 9.2	619^{ac} ± 9.1	-0.65^{abc} ± 0.85	-40.9 ± 6.95
	2008	66	671^{a} ± 9.3	640^{a} ± 8.7	0.38^{abc} ± 0.93	-33.7 ± 7.26
	2009	73	660^{a} ± 8.5	613^{ac} ± 8.5	-1.72^{c} ± 0.81	-41.2 ± 6.59
	2010	61	662^{a} ± 9.2	603^{c} ± 9.2	0.26^{abc} ± 0.90	-47.9 ± 7.27
	2011	1	705^{ab} ± 63.2	655^{abc} ± 59.6	0.27^{abc} ± 4.30	-48.4 ± 47.8

*Different superscripts within factor denote significant difference at $p < 0.05$

Body weight at drying, calving and the rate of change in body weight during the first 15 days of the dry period was not significantly different between cows that did not develop clinical disease and those that did develop disease in the *post* parturient period. However, cows that developed reproductive disorders in the first 30 days of lactation had lost significantly ($p < 0.001$) more weight in the dry period than those cows with no clinical disease and those that developed mastitis. Cows that developed metabolic diseases did not have significantly different changes in body weight from any of the other health groups.

Year had a significant effect on body weight at drying off, calving and the rate at which body weight changed in the first 15 days of the dry period. In the years 2004 and 2005, cows were significantly heavier at drying than in the years 2006 to 2010 inclusive. Body weight at drying, calving and the rate of body weight change in the first stage of the dry period in 2003 were not significantly different to these traits as measured in 2011. Higher standard errors in years 2003 and 2011 can be explained by smaller sample size. Only 3 months of each of these years were included.

3.4.4 Traits from the change-over period

MBER was significantly ($p < 0.001$) affected by production system, parity, health group and year of production. Lsmeans and standard errors for MBER with respect to each of the fixed factors are presented in Table 3.4.

Low Forage Control cows had an MBER of 0.91, significantly greater than that of High Forage Control cows (0.68). Cows moving to second lactation had significantly ($p < 0.001$) higher MBER (0.82) than those moving to third lactation (0.73). Cows that went on to develop mastitis in the first 30 days of lactation had a significantly higher MBER (0.92) than cows which did not develop clinical disease and those that developed reproductive disorders ($p < 0.05$). Average MBER was significantly lower in 2004 than in years 2005 – 2010, inclusive. Milk yield at dry off was significantly affected ($p < 0.001$) by production system but was not affected by parity. Health group ($p = 0.035$) and calendar year ($p < 0.01$) had a significant effect on milk yield at dry off.

Table 3.4: LSMeans with standard errors and p-values for milk yield: body energy content ratio and yield at dry off by system, previous parity, health group and year

		MBER	**Yield at Dry Off (litres)**
Factor	**Level**	**LSMean ± SEM**	**LSMean ± SEM**
System	Low Forage Control	0.91 ± 0.061^{a}	23.6 ± 1.06^{a}
	Low Forage Select	0.81 ± 0.059^{ab}	18.4 ± 1.04^{bc}
	High Forage Control	0.68 ± 0.057^{b}	19.2 ± 1.00^{b}
	High Forage Select	0.68 ± 0.058^{ab}	16.3 ± 1.02^{c}
Parity	1	0.82 ± 0.050^{a}	19.6 ± 0.89
	2	0.73 ±0.051^{b}	19.2 ± 0.89
Health Group	No clinical disease	0.81 ± 0.044^{a}	19.9 ± 0.78^{ab}
	Subclinical mastitis	0.92 ± 0.059^{b}	21.3 ± 1.08^{a}
	Reproductive	0.74 ± 0.055^{a}	18.6 ±1.00^{b}
	Metabolic	0.70 ± 0.093^{ab}	17.8 ± 1.69^{b}
Year	2003	0.59^{ab}	14.3 ±2.01^{ac}
	2004	0.53^{b}	15.6 ± 0.93^{c}
	2005	0.77^{a}	19.4 ± 0.79^{a}
	2006	0.78^{a}	19.4 ± 0.82^{a}
	2007	0.86^{a}	20.7 ± 0.85^{a}
	2008	0.79^{a}	20.8 ± 0.80^{b}
	2009	0.87^{a}	21.5 ± 0.79^{b}
	2010	0.87^{a}	20.3 ±0.86^{a}
	2011	0.86^{ab}	22.5 ± 6.19^{ac}

*Different superscripts within factor denote statistical significance at $p < 0.05$

Low Forage Control cows had a significantly greater ($p < 0.05$) dry-off yield than cows from all other systems. High Forage Select cows had significantly lower ($p < 0.05$) dry-off milk yield 16.3 (s.e.m = 1.02) litres per day) than High Forage Control and Low Forage Control cows. Of the cows that developed disease in the first 30 days of lactation, those that developed mastitis had significantly higher yields (21.3 litres per day) than those that developed reproductive or metabolic conditions.

3.5 Discussion

3.5.1 Production System

Production system had a significant effect on the rate of change in body weight, body condition score and body energy content in the first two weeks of the dry period. Irrespective of genetic merit, cows fed a low forage diet in the previous lactation mobilised reserves throughout the dry period, whereas cows previously fed a high forage diet gained reserves.

Differences in the rate of change of body weight in the first two weeks of the dry period between control cows fed a low or high forage diet may be explained by changes in gut fill. Diet type is known to influence gut fill and its effect on liveweight has presented a challenge in several other studies, particularly in those examining the effects of forage type and inclusion. Imani *et al*. (2017) found that gut fill was a confounding effect when evaluating weight gain in dairy calves fed a high forage diet. Similarly, Kahyani *et al*. (2019) who investigated the effect of forage type on cow performance, were not able to rule out that differences in body weight gain seen between dietary treatments were due to changes in gut fill, potentially caused by differences in dry matter intake. It was not possible to measure dry period dry matter intake in the current study but Ross *et al*. (2014), reported no significant differences in daily dry matter intakes in lactation amongst the four production systems throughout the same long term study, although select cows fed a low forage diet had the numerically highest daily dry matter intake (20.2 kg). The decreased nutrient demand of a dry cow means that the dry matter intake of non-lactating cows is significantly lower than those in lactation, it may therefore be assumed that gut fill will decrease substantially as cows move from a lactation diet to a dry period diet. However, when the dietary change involves a large increase in forage intake, as experienced by cows moving from a low forage lactation diet, the relationship between dry matter intake and gut fill may not be straightforward. Su *et al*. (2017) reported that the inclusion of lower quality feedstuff (i.e. those with a greater fibre content) reduces dry matter intake because of a reduced rumen outflow rate and digestibility and therefore gut fill increases.

The most detailed analysis of the effect of gut fill on body weight changes is that of Thorup *et al*. (2012; 2013; 2018) as part of their approach to modelling energy balance dynamics in dairy cattle. Of relevance to the current study, variation in meal-related gut fill was not found to differ according to diet type (TMR vs. grazing). Additionally, when their data smoothing procedure resulted in a 4 kg difference between actual and predicted gut fill, its effect was ignored thereby suggesting that its influence on the calculation of body weight and energy balance is relatively minor (Thorup *et al*., 2018). Further, Thorup *et al.* (2018) used data from lactating cows where a much larger variation in gut fill would be expected relative to dry cows eating a maintenance only diet.

Without accurate dry matter intake data, it is not possible to fully understand the effect that gut fill had on changes in liveweight in the current study. However, in the context of previously published work, gut fill is likely to have only had a small influence on the accuracy of body weight and body energy calculations and its influence is likely to be similar across production systems irrespective of diet. That changes in body condition score were also seen between production systems in the first two weeks of the dry period, suggests that energy status was being affected beyond temporary gut fill related changes. Dry matter intake of transition cows has been measured in a number of published studies; Mann *et al*. (2015) reported intakes of between 14.2 and 16.4 kg per cow per day in the last 28 days before predicted calving however, it is important to note that these diets included feedstuffs which are not permitted for use in the United Kingdom, including monensin and blood meal. In contrast, Friesian cows managed in a forage-based system, and ranging between 610-632kg liveweight, had mean DMI of between 10.8 and 12.3 kg/cow/day (O'Driscoll *et al*., 2009). In a study of 101 Holstein cows, in the 2 weeks before calving, healthy cows were found to consume between 13.8 kg/day and 15.4 kg/day (Huzzey *et al*., 2007). Diet presentation has also been reported to affect intake in transition cows – cows fed a diet with a total DM content of 45.2% consumed an average of 14.2kg/day compared to cows fed a diet with a total DM of 53.4% that consumed 13.3kg/day (Havekes *et al*., 2020). Additionally, Eslamizad *et al*. (2015) demonstrated that pre-partum cows reduced feed intake in response to heat stress by decreasing meal size, meal duration, eating rate and daily eating time.

Another dramatic dietary change that may also account for the initial mobilisation of body reserves is the abrupt dietary change that low forage cows were subject to when they were moved from a lactation diet composed of 45% forage, to a dry period diet composed of 95% forage. It is well documented that any dietary change experienced by a ruminant requires a shift in the rumen population and adaptation of the ruminal papillae (Sun *et al*., 2019). Derakhshani *et al*. (2017) reported an increased number of proteolytic, amylolytic and lactate-producing species of rumen bacteria and fewer fibrolytic bacteria in the rumen fluid of *postpartum* (lactating) dairy cows compared to *prepartum* (dry) cows. If the converse of this is true, then cows fed a low forage lactating diet would not have the optimum rumen conditions (i.e. enough fibrolytic bacteria) to deal with a high forage dry period diet. Therefore, it can be expected that the sudden increase in dietary forage content will necessitate a significant change in rumen biology, which will lag the dietary change and may explain the mobilisation of energy reserves in the first 15 days of the dry period as the rumen adapts. Derakhshani *et al*. (2017) suggest that acclimatisation of rumen microorganisms during the peripartal period can facilitate the transition into lactation. Acclimatisation of rumen microorganisms to a typical dry cow diet in late lactation may therefore be beneficial in facilitating the transition from lactation to the dry period, in order to avoid body energy mobilisation in the early dry period, such as was seen in the current study. Additionally, Sun *et al*. (2019) in their review hypothesise that the change in the rumen microbiome necessitated by sudden diet changes may have additional consequences, as it directly alters the supply of nutrients provided for other tissues, organs and their corresponding functions including implications for inflammation, oxidative stress and immune function which are of vital importance in the dry and transition periods.

The relationship between body energy change in the early dry period and production system may also be explained by the significantly greater “starting” body energy content and body condition of cows fed a low forage diet compared to those fed a high forage diet. In the current study, cows fed a low forage lactation diet had significantly greater energy reserves at the time of drying (3443±133.1 MJ & 3340±140.5 MJ) compared to those fed a high forage lactation diet (2908±135.7 MJ & 2808±138.3 MJ) but mobilised more reserves over the dry period compared to those fed a high forage diet. Energy status at drying off is directly associated with the change in energy status throughout the dry period. Differences in body energy content at the end of lactation when cows are dried off have been previously reported; Roche *et al*. (2017) found that cows from pasture-based systems are generally thinner at the end of lactation than cows fed total mixed rations. Relative to the association between "starting body energy content" and subsequent change in energy status, Garnsworthy and Topps (1982) demonstrated that cows with a higher condition score at calving lost more weight and condition in early lactation than cows of modest condition at calving. This relationship was further investigated by Broster and Broster (1998), who found that over-conditioned cows experienced more rapid mobilisation of body energy reserves in early lactation than those in optimum condition at calving. During lactation, cows can be forced from their natural body energy cycle by environmental factors specific to the lactation period, whereas in the dry period when milk production ceases and management is less intensive, cows have the opportunity to modulate their body energy reserves according to their genetic disposition. In the current study, higher body energy content at dry off appears to be associated with a greater loss in condition in the early dry period. Higher body energy content at the end of lactation (dry off) may have a similar effect on ensuing changes in body energy content as is seen with higher body energy content at the initiation of lactation (calving).

Prior to assignment to one of the two feed systems at the time of first calving, all animals in the current study were managed together. With reference to data from the same long-term experiment, Coffey *et al*. (2006) *post*ulated that any differences in growth rates prior to calving could solely be attributed to differences in genetics. They went on to suggest that because control cows tended to be at a "weight disadvantage" at calving, *post*-calving compensatory weight gain would occur which would have a negative effect on milk yield. As such, it was expected that genetics on their own would exert a significant effect on patterns of change in body energy and liveweight throughout the dry period, but this was not seen in the current study.

However, at the time of dry-off, production system did have a significant effect on the ratio of milk produced in relation to body energy content. Cows from the Control line fed a low forage diet produced 0.91 litres of ECM per day per 100 MJ of body energy content, significantly higher than the 0.68 litres produced by control cows fed a high forage diet. Friggens and Newbold (2007) state that the implications of genetic x environment interactions are that some genotypes are better suited to meeting environmental challenges. In the current study, significant differences exist between cows of the same genotype (Control genetic line) subject to different environmental conditions. Cows of the same genetic line fed a more energy dense diet (i.e. low forage) would be expected to produce a higher total daily milk yield than those fed a lower energy diet (i.e. high forage). Indeed, throughout the study period, control cows fed a low forage diet had an average daily yield of 30.5 kg/day compared to an average daily yield of 23.9 kg/day for control cows fed a high forage diet. MBER however represents yield per 100 MJ of body energy reserves, and therefore is standardised across different total daily milk yields. Although from the results of this study it cannot be concluded that genotype on its own affects the ability of cows to respond to environmental challenges (e.g. a poorer quality diet), the potential for interactions between genetics and environment warrants further investigation.

3.5.2 Parity

As anticipated, parity had a significant effect on measures of body energy content in accordance with the existing literature. Cows completing second lactation had a body energy content of 2929 ± 118.6 MJ compared to 3353 ± 119.6 MJ for cows completing third lactation. Coffey *et al*. (2002) showed that the dynamics of body energy content in first lactation animals were different to that of cows in later lactations. Similarly, Dewhurst *et al*. (2002) reported that the continued requirement of first lactation animals to grow influences nutrient requirements and partitioning during first lactation and possibly subsequent lactations. It is logical to assume that growth will be a priority and continue to influence nutrient requirements and partitioning until mature size is attained, irrespective of lactation number. If, in an attempt to reduce age at first calving, heifers begin first lactation further away from their mature weight then the nutritional requirement for growth may extend into second or even third lactation. In the current study, the significant difference between liveweight of cows completing first lactation and cows completing second lactation indicates that growth continued throughout second lactation. This presents a particular challenge for young cows as to allow for this growth, energy will have been partitioned away from other pathways e.g. milk production and/or pregnancy.

Carry-over effects of body energy content from one lactation to the next should also be considered. Coffey *et al*. (2001) reported that body energy mobilised in early lactation was on average not fully recovered until day 200 of lactation. Failure to regain lost energy reserves before the start of the following lactation resulted in a life-long downward trajectory of body energy content, as the deficit to be replenished in each subsequent lactation increased. In addition to these reported cumulative effects, there is evidence which suggests that residual effects also exist between lactations. Although they acknowledged the conclusion of Oldham and Emmans (1989) that sustaining the current lactation is a higher priority than accumulating body reserves, Dewhurst *et al*. (2002) reported that once lactation potential is lost, due to underfeeding, it is not recovered in the next lactation even when additional dietary energy is provided - instead the extra energy is directed to body reserves. Further research on parity and feed system/nutrition interaction is required to disentangle this relationship. The power of this study to do so is limited in that all animals were fed the same dry cow diet for the first 6 weeks of the dry period which may have ameliorated any residual effects of nutrition in the previous lactation, as reported by Dewhurst *et al*. (2002). Due to the important effects that parity has on body energy and liveweight, as outline, its effect must be accounted for in any attempt to model body energy content with a view to identifying cows at risk of disease.

3.5.3 Calendar Year

No consistent year-on-year trends were detected in body weight, condition score or energy content across the study period although some significant differences between individual years exist. Caution must be employed when interpreting results for 2003 and 2011 as sample numbers in these calendar years were low due to start and end dates of the long-term study from which the data was sourced. Body energy content at dry-off was significantly greater in 2004 than in all other calendar years, except 2011. This is potentially a legacy effect from the previous management systems to which these cows were subject, with no effect being detected in 2003 due to the small sample size for that year.

Further, weather mediated changes in grazing conditions and forage quality are a likely cause for some of the individual year differences. Cows in the high forage system were grazed when weather conditions permitted – differences in rainfall during the summer months therefore determined the length of the grazing season each year. Sub-optimal grass growth due to poor growing conditions also influenced the length of the grazing season as well as determining forage quality, which was important for both cows fed low and high forage diets. Reduced forage quality had implications on the choice of concentrate feed supplementation required to meet the diet specification outlined in Chapter 2. Regional and global influences on feed prices related to specific years or weather conditions also exerted an influence on the choice of concentrate feed supplementation used. Due to the indirect effects that calendar year has been seen to have on physiological cow parameters, it was correct to include it in the model, however such data would not be available for use in a real-time model used in a disease detection aid. Continuous local climate data may be a valuable addition to a real-time model.

3.5.4 Health Status

Cows that lost body energy content at the highest rate during the first fifteen days of the dry period went on to develop reproductive disorders in the *postpartum* period. Cows that developed retained placenta or metritis lost on average 18.26 MJ of body energy per day for this 15-day period, whilst cows that did not develop clinical disease gained an average of 0.63 MJ per day. In relation to body condition, it has previously been reported that incidence of metritis and metabolic diseases in early lactation was significantly greater amongst cows that had a marked loss (1 – 1.5 points) in condition in the dry period compared to those with moderate loss (0 – 0.75) (Kim and Suh, 2003). Gearheart *et al*. (1990) found that cows that lost the most condition in the dry period were at an increased risk of developing dystocia and being culled in the subsequent lactation. Similarly, Markusfeld *et al*. (1997) reported that cows that lost most condition during the dry period had an increased risk of retained placenta and metritis. In contrast to the results of the current study and to Gearheart *et al*. (1990), Markusfeld also reported a significant relationship between body condition at dry off and condition change in the dry period.

In terms of body weight, cows that developed reproductive disorders lost 55% more body weight during the dry period than cows that remained healthy after calving. Given that there was no significant difference in body weight, body condition or energy content of cows with no clinical disease and those that developed reproductive disorders at dry-off or calving, it would appear that it is the change in these traits during the dry period that exert an influence on future disease, risk rather than the absolute level of each or any of the individual traits. Garnsworthy (2006) argued that the rate of body energy mobilisation may be of greater importance in managing the risk of disease in the transition period rather than absolute over-conditioning, contrary to previous thinking. The results from the current study appear to support this argument. Rapid mobilisation of energy reserves causes physiological stress which manifests itself in suppressed dry matter intake and milk yield in early lactation, alongside an increased incidence of health and reproductive problems (Roche and Berry, 2006). Interestingly, Huzzey *et al.* (2007) found that for every 10 minute decrease in average daily feeding time during the week before calving, the odds of severe metritis increased by 1.72 and for every 1 kg decrease in dry matter intake, cows were nearly 3 times more likely to be diagnosed with metritis in the first three weeks of lactation. In their study, cow behaviour monitoring *prepartum* was only performed in the two weeks before calving – could this effect begin much earlier in the dry period and explain the mobilisation of body energy as seen in the current study?

The finding that cows which developed *post*-calving reproductive disorders exhibited significantly different traits in the early dry period highlights the importance of the "far-off" dry period and the relevance of studying the whole dry period when considering disease risk in the following lactation. Many studies which have identified risk factors for *postpartum* disease, including those cited above (Gearheart *et al.*, 1990; Markusfeld *et al*, 1997, Kim and Suh, 2003; Roche and Berry, 2006) have not specifically focussed on the early dry period. Although of unrivalled importance, attention should not focus solely on the transition period around calving, but also on the change-over from lactation to the dry period since cows also undergo significant physiological change during this "first transition". Nutritional management during this stage has been shown to significantly affect physiology in early lactation. Dann *et al.* (2006) reported that cows fed lower energy diets in the far-off period (80 and 100% of energy requirement) had greater dry matter intake and energy balance in the first 10 days of lactation compared to cows overfed energy (150% of requirement). The carryover effect from the far-off diet diminished as lactation progressed. Although Mann *et al.* (2015) found no significant effect of dietary energy level throughout the dry period on *postpartum* milk production or dry matter intake, they did report a higher incidence of ketosis amongst cows fed a diet exceeding energy requirements during the whole dry period as well as in cows fed a controlled energy diet in the far-off period only.

In the current study, high milk yield at dry off was associated with early lactation subclinical mastitis. It has previously been reported that cows which have not had a significant reduction in milk yield before dry off have higher levels of intramammary infection compared with cows whose daily yield had reduced in the period before dry off, although the optimal level of production at dry off is not clear (Dingwell *et al*., 2001). Martin *et al*. (2020) summarised the risks associated with abrupt dry-off of cows maintaining high yields in late lactation as; increased risk of intramammary infections during the dry period and at calving and a higher somatic cell count in the subsequent lactation. Most epidemiological studies of mastitis, including that of Dingwell *et al*. (2001), focus mainly on dry period acquired environmental infections. However, in the current study, no distinction was made between cases of persistent infection and cases of dry period acquired infection. Like milk yield, high MBER was associated with a diagnosis of subclinical mastitis in early lactation, meaning that at the end of lactation, these cows were producing more milk relative to their body energy content. In theory, this may indicate that cows with a high MBER value had different nutrient partitioning priorities i.e. prioritising milk yield over modulating their body energy reserves.

No significant differences were found between cows that remained healthy and those that developed metabolic disorders in early lactation across any of the traits examined. It is possible that this result may have been skewed by the very small sample size of cows diagnosed with metabolic disease (n=17), due to the high level of management that was maintained throughout the long-term study. This necessitated that all metabolic disorders (hypocalcaemia, hypomagnesaemia, left displaced abomasum and ketosis) were grouped together, despite each disease having distinct epidemiology. It is important to bear in mind that as an observational study, one major advantage is that all disease incidences were naturally occurring and therefore are likely to resemble disease events on commercial farms.

An important finding of the current study is that different body energy dynamics are experienced at critical timepoints in the lactation-gestation cycle between cows that go on to develop different diseases. Although absolute body weight, condition score and energy content at dry off and calving were not able to distinguish between health groups in this study, their value cannot be entirely dismissed due to the extensive evidence from studies suggesting that over or under condition does play a crucial role in determining disease risk. In the current study, there was not an extreme range of any of these traits which may have affected the power to assess the effects of true over and under-conditioning on disease risk. It must be acknowledged that the findings of this study may have been different under different herd size, management or feeding systems However, although data used was sourced from one farm, the four dairy production systems in operation represented contrasting approaches to dairy herd management and reflected a range of farming systems (Ross *et al*., 2004). Inclusion of the production system in the analysis allowed the effect of genotype and environment to be accounted for in addition to other factors. Further, the rich longitudinal nature of the dataset afforded the opportunity to access data for individual cows for an extended period, throughout which all aspects of management and production were recorded.

This study has demonstrated that cows that develop different production diseases in early lactation exhibited some difference in physiological characteristics both at the end of the previous lactation and throughout the dry period. These results provide initial support for the inclusion of measures of MBER, the rate of change in body energy content during the early dry period and the difference in body weight, condition and energy content in disease prediction and detection models, the need for which has been clearly identified by a number of previous researchers e.g. Ingvartsen *et al*. (2003), Rutten *et al*. (2013). The complexities of the relationship between energy balance and early lactation disease mean that any statistical model used to predict disease risk must control for a range of explanatory factors (e.g. parity) and the interdependence between risk factors. Further work is required to establish the viability of including any of these measures in predictive modelling including examining the distributions of each parameter within healthy and sick groups to establish their discriminatory power.

Of particular relevance to the future development of on-farm tools, all the measures used in the current study are non-invasive and can conceivably be recorded on commercial farms, particularly those using voluntary milking systems. A challenge exists as to how best record condition score and live weight data in the dry period where cows are less intensively monitored and except for calving alert devices, few precision livestock tools currently exist for use in this period.

3.6 Conclusion

Cows that developed different diseases or had no clinical disease in the first thirty days of lactation exhibited different physiological traits at the end of the previous lactation and throughout the dry period. Of particular interest is that cows that developed reproductive disorders in early lactation mobilised significantly greater body energy reserves in the first 2 weeks of the preceding dry period compared to all other cows. The current study has thus identified the transition from lactation to the dry period as being of critical importance in maintaining animal health and influencing future disease risk and productivity. Furthermore, the results of the current study have important implications for the inclusion of on-farm data in models for the prediction of disease risk and disease detection. Production system and parity exert significant effects on body energy content, live weight and condition score both at distinct time points in the lactation-gestation cycle and on changes in these traits over time. Modelling of these traits to allow for identification of at-risk animals must therefore be sensitive to and account for production system. Improved methods of data handling and analysis are required to make best use of routinely recorded "on farm" data in the development of precision farming tools, which can then be utilised to ultimately reduce disease incidence and improve efficiency on farm.

Chapter Four

Developing models to identify individual cows at risk of early lactation disease

4.1 Introduction

Veterinary examination is, and will continue to be, the gold standard for disease detection on dairy farms, however, the infrequency of these examinations and the adoption of precision livestock farming tools mean that an opportunity exists to exploit the wealth of data currently recorded on commercial farms to identify individual cows at risk of developing disease (Urton *et al*., 2005; Rutten *et al*., 2013). Identifying individual cows or cohorts at risk of disease as early as possible would allow time for interventions to reduce disease incidence (Wisnieski *et al*., 2019d). It is undisputed that a practical method for assessing health status and risk of disease amongst dry and transition cows would be beneficial (Ingvartsen *et al*., 2003; Urton *et al*., 2005).

Difficulties exist in using production data as a means of identifying cows at-risk due to the complex inter-relationship between yield and disease, as discussed in Chapters 1 & 2. Moreover, the period of highest disease incidence in the lactation-gestation cycle (early lactation), when the identification of at-risk cows would be of most value, is preceded by the dry period when cows are not producing milk and are less intensely monitored than during lactation. These two challenges mean that historically, assessment of health status and "transition period success" has relied on the use of outcomes with a significant lag, such as peak milk yield or culling/survival rate at 60 days in milk (Schultz *et al*., 2016). A more sophisticated approach to assessing transition period success – the Transition Cow Index (TCI), was developed by Nordlund and Cook in 2004. Although there still is a lag effect, the advantage that this method has over simply looking at peak milk yield is that it combines a variety of cow-level data (previous 305-day milk yield, days in milk in preceding lactation, start of current/preceding lactation as calving or abortion, month of calving, somatic cell count at last test of preceding lactation, days dry, milk frequency in current lactation, milking frequency in preceding lactation, parity, breed and bovine somatotrophin use) and calculates an expected 305-day projected milk yield following the first milk test after calving (Nordlund and Cook, 2004). The TCI is calculated as the difference between the projected and actual first test 305-day projected milk yield. Results of an unpublished study using TCI reported that a TCI of 450 litres was associated with 580 litres of additional cumulative milk yield, 130 litres greater than the predicted 450 litres (Nordlund, 2012). Relative to health outcomes, Brotzman *et al*. (2015) found that high TCI was associated with improved udder health, but no published literature exists which relates TCI to the incidence of metabolic disease. The main limitations of the TCI method are the lag effect (with standard herd testing protocols, individual cows may not have their first milk test until 40 days in milk) and the fact that it cannot be calculated for primiparous cows or cows that leave the herd before their first milk test (Schultz *et al*., 2016). Although the TCI is an excellent tool for evaluating transition period success, the opportunity for it to be used as a means of identifying at risk cows and allowing early interventions to prevent disease is somewhat limited.

An additional challenge in the development of a monitoring system for transition cow health status is that in most instances it is still unclear which parameters of routine herd data are the most appropriate indicators of disease, particularly in the dry and transition periods (de Vries *et al*., 2011). Thus, most studies have focused on monitoring systems for use in lactation e.g. using milk-based parameters to detect mastitis, activity monitors for the detection of oestrus etc. Sensor systems for diseases typically associated with the dry and transition periods have not been as extensively researched (Rutten *et al*., 2013). Therefore, the identification of physiological measures in the dry period which are strongly related to disease risk in early lactation is crucial to the further development of automated means of monitoring health status and disease risk (Huybrechts *et al*., 2014; Lukas *et al*., 2014).

Van Dixhoorn *et al*. (2018) developed and tested a statistical model to predict disease severity and "resilience" in early lactation which included data from activity and behaviour monitors and rumen and ear temperature sensors, recorded from two weeks pre to 6 weeks *post* calving. During this period a score (total deficit score – TDS) was calculated by clinically assessing each cow daily to quantify disease length and severity before the sensor data recorded during the transition period was tested as a predictor for TDS (van Dixhoorn *et al*., 2018). In line with similar studies in lactating cows (Liberati and Zappavigna, 2009; Højsgaard and Friggens, 2010), the integration of data from several different monitoring systems enhanced the predictive accuracy of the model. The optimal linear combination of variates, with the lowest prediction error, was average eating time, variance of daily ear temperature and regularity of daily behaviour patterns – meaning that cows at higher risk of disease had less consistent behaviour patterns. Although their study provided proof of concept for the use of time-series data to predict health status in dry and transition cows, the low number of cows in the study (n = 22) means that the results achieved should be taken with caution. Additionally, integration of different blood biomarker data has also improved the goodness of fit of predictive models for cohort-level disease risk (Wisnieski *et al*., 2019a). Moving on from their proof of concept study, Wisnieksi *et al*. (2019b) found that aggregating data from the individual level to the group level allowed prediction of early lactation health events at dry off thus allowing time to implement proactive interventions in a group of cows projected to have a high disease incidence.

More recently, measures of cows feeding and competitive behaviours before calving have been successfully used to predict which cows are likely to develop metritis, ketosis and mastitis using predictive models which achieved sensitivities of 71-73% and specificities of 80-84% (Sahar *et al*., 2020). Separate models were developed for primi- and multiparous cows. For multiparous cows, higher *prepartum* body weight and increased "actor" behaviour (the number of times which a cow replaces another cow at the feed barrier) increased the odds of *postpartum* disease (Odds Ratio = 1.31 and 2.47 respectively) (Sahar *et al*., 2020).

Apart from the literature outlined above, relatively few studies have used modelling to predict early lactation disease. Rather, the majority have focussed on explanatory modelling i.e. testing the causal relationship between predictors and health outcomes (Wisnieski *et al*., 2019d). There is a need to develop and test predictive models based on the knowledge gained through explanatory modelling and using predictors that are biologically relevant and improve predictive ability. In this context, the analysis conducted in Chapter 4 marks a progression from Chapter 3 which sought to answer the question as to whether dry period measures differed between groups of cows with different health outcomes in subsequent lactation. In the current study, the capability of dry period measures (either singly or in combination) to predict disease incidence level in subsequent lactation will be evaluated.

4.2 Hypothesis & Objectives

The hypothesis for the current study was that indicators of early lactation disease identified as being significantly different between cows of different health status after calving would be of value in predictive modelling to identify cows at greater risk of disease in the 30 days after calving. This was tested by screening the distributions of each potential predictor according to cow health status and testing the inter-relationships between the potential predictors before developing a predictive model. The value of each of these potential predictors in predicting early lactation disease was investigated under the null hypothesis that they would not be able to improve the predictive ability of a model built to identify cows at increased risk of disease.

4.3 Materials & Methods

4.3.1 Data source

Data were obtained for this study from the herd of Holstein-Friesian cattle at Scotland's Rural College's (SRUC) Dairy Research and Innovation Centre, Crichton Royal Farm, Dumfries. Data used were from a period of eight years, from November 2003 to September 2011, when cattle were on a long-term 2x2 factorial experiment which investigated the interaction between genotype and environment (Pryce *et al*., 2001). For full details of herd management and data collection see Chapter 2 Materials & Methods.

4.3.2 Data handling

Cow-lactations were used as the experimental unit throughout the analysis. The total number of cow-lactations eligible for use in this study was 505, from 342 individual cows moving between lactations 1 and 2, and between lactations 2 and 3.

4.3.2.1 Classification of cow-lactations

In Chapter 2, each of these cow-lactations were assigned to 1 of 4 groups based on disease incidence in the first 30 days of the on-going lactation. These groups were: no clinical disease (NCD), reproductive (REP), mastitis (MAST) and metabolic (MET) (see Table 2.7 for classification criteria). For the purposes of this analysis, these disease classifications were used to create 4 binary responses for each of the three disease categories individually and one for all diseases combined. This was to allow for a greater number of cow-lactations to be used in each analysis e.g. rather than only comparing cows with reproductive disorders to cows with no disease, cows with reproductive disorders could be compared to all cows with no reproductive disorders. Additionally, previous disease history was considered by creating binary measures based on occurrence of the 3 disease classes in the previous lactation.

4.3.2.2 Candidate indicator traits

A total of 20 candidate indicator traits were identified and evaluated in the analysis; 7 traits were brought forward from Chapter 3 and 12 new traits were created as detailed in Table 4.1. Body weight (kg), condition score and body energy content (MJ) recorded at dry of and calving and recorded weekly from the day of dry off until the day of calving were extracted for each cow-lactation (Ind 1 – Ind 6). Body energy content was calculated using the method given by Banos *et al*. (2006) utilising the NRC equations (see Chapter 3 Materials & Methods). Body weight and body energy content were subject to basic filtering to eliminate erroneous data. Liveweight data were ignored if two consecutive measurements were greater than 140kg apart.

Table 4.1: Candidate indicator traits used in Chapter 4 analysis

Abbreviation	Trait	Description	Type/Source
Ind1	LW at dry off	Live weight on day of dry off (kg)	Chapter 3
Ind2	CS at dry off	Body condition score on day of dry off (CSU)	Chapter 3
Ind3	LW at calving	Live weight on day of calving (kg)	Chapter 3
Ind4	CS at calving	Body condition score on day of calving (CSU)	Chapter 3
Ind5	BEC at dry off	Body energy content on day of drying (MJ/day)	Chapter 3
Ind6	BEC at calving	Body energy content on day of calving (MJ/day)	Chapter 3
Ind7	BW intercept (calving)	Intercept from linear regression of body weight versus days from calving on day of calving	New
Ind8	BCS intercept (calving)	Intercept from linear regression of condition score versus days from calving on day of calving	New
Ind9	BEC intercept (calving)	Intercept from linear regression of body energy content versus days from calving on day of calving	New
Ind10	BW intercept (-60)	Intercept from linear regression of body weight versus days from calving at 60 days before calving	New
Ind11	BCS intercept (-60)	Intercept from linear regression of condition score versus days from calving at 60 days before calving	New
Ind12	BEC intercept (-60)	Intercept from linear regression of body energy content versus days from calving at 60 days before calving	New
Ind13	BW Slope	Gradient from linear regression of body weight versus days from calving	New
Ind14	BCS Slope	Gradient from linear regression of condition score versus days from calving	New
Ind15	BEC Slope	Gradient from linear regression of body energy content versus days from calving	New
Ind16	Yield at dry off	Total daily milk yield on day of dry off (litres)	Chapter 3
Ind17	Previous REP	Binary measure for incidence of reproductive disease in previous lactation (0/1)	New
Ind18	Previous MAST	Binary measure for incidence of subclinical mastitis in previous lactation (0/1)	New
Ind19	Previous MET	Binary measure for incidence of metabolic disease in previous lactation (0/1)	New
Ind20	Previous ANY	Binary measure for incidence of any disease in previous lactation (0/1)	New

Body energy content data were ignored if two consecutive measurements were greater than 700 MJ apart. This was performed in order to eliminate very obvious data errors which are known to arise if a cows identification tag was not read correctly on the walk over weigh scale, or if a fault allowed more than one cow to be on the weighing platform simultaneously. Regression analysis was performed on weekly BW, BCS and BEC data to model these traits across the dry period and to calculate the intercept at calving and at 60 days before calving (Ind 7 – Ind 12) and gradient (the slope or rate of change in BW, BCS or BEC) according to days from calving (Ind 14 – 16).

4.3.3.3 Statistical analysis

Statistical analysis was performed in GenStat employing PROC GLMM.

(i) Exploring distributions

Histograms of data for each variable (both categorical and continuous) were plotted to examine their distributions and to check for normality, skewness and outliers. Outliers were identified in order to ensure that results were not unduly affected by outlying data points however, they were not eliminated as it was assumed that some of these extreme values would be indicative of cows suffering from disease (i.e. what the model was looking for). Dry period length was standardised by subtracting the mean and dividing by the standard deviation and then taking the absolute value of this (i.e. ignoring the positive or negative sign). This transformation was applied because it had been observed that health problems appear to occur at the extremes (positive or negative) of the distribution of the variables on the original scale (see Figure 4.1 for an example).

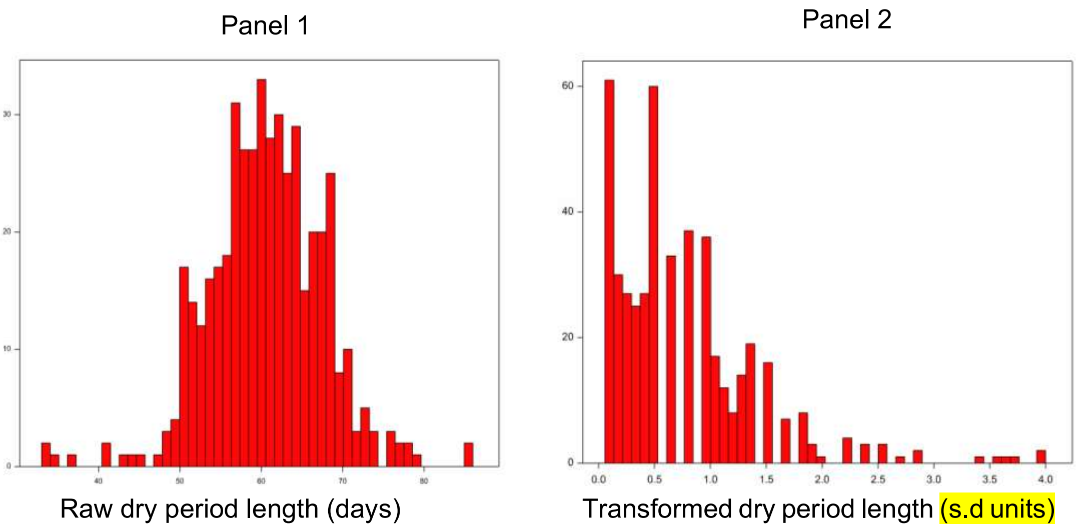


Figure 4.1: Histogram showing effect of transformation on distribution of dry period length (days)

Distributions of each variable were also plotted according to each response measure to visually assess the discriminatory power of each variable (see Figure 4.2 for an example).

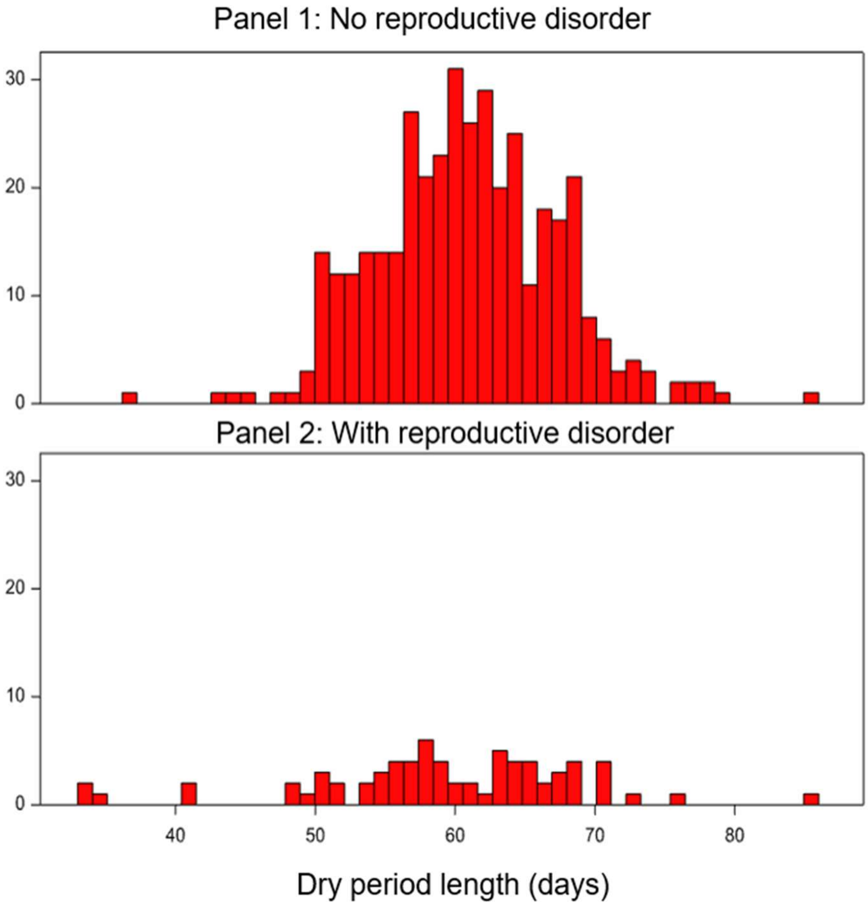


Figure 4.2: Distribution of dry period length in days for cows with no reproductive disorder in the first 30 days of lactation (Panel 1) and cows with a reproductive disorder (retained placenta or metritis) in the first 30 days of lactation (Panel 2)

Cross-tabulation of explanatory categorical variables with response measures identified sparse data due to low incidence of metabolic disease. Thus, it was not possible to include year or month of calving as an explanatory variable in a Generalized Linear Mixed Model for metabolic disease.

(ii) Understanding interrelationships

A series of pairwise statistical tests was performed on the data to screen for relationships between all variables (health response measures and candidate indicators). Linear relationships between continuous variables were tested using Pearson correlation tests and Spearman correlation tests were used to test relationships between ordinal variables, based on ranked values. Correlation coefficients can range in value from -1 to +1. The larger the absolute value of the coefficient, the stronger the relationship between variables. A coefficient of +1 is indicative of a perfect positive correlation, as one variable increases, the other increases by a proportionate amount whereas a coefficient of -1 indicates a perfect negative correlation; if one variable increases, the other decreases by a proportionate amount (Field *et al*., 2012). A coefficient of 0 indicates no linear relationship

Relationships between factors were tested using a Chi-square permutation test, which is a nonparametric alternative to the more usual chi-square test of independence which is unreliable with smaller numbers and is therefore not appropriate for use in this study. The test works based on a randomised non-theoretical simulation exercise which creates a series of cross-tabulations from the data and compares the observed value to the distribution of permuted data, relative to the null hypothesis. ANOVA was used to investigate relationships between continuous variables and factors by testing the difference in means between groups.

(iii) Generalised linear mixed modelling

Generalised linear mixed modelling (GLMM), fitted with logit link assuming binomially distributed errors, with random effect for cow to allow for variability between cows as well as between response measures on the same cow, was used to test the effect of candidate indicator variables on each response measure. GLMM was used, as unlike conventional linear models, it allows for response variables from different distributions e.g. binary responses. The logit link function was used to convert covariates to the probability scale. The probability of a health problem was estimated averaging over the cow random effects because the effect due to each cow would not normally be available in practice when making true predictions. Continuous variables were standardised to assist model fitting. Firstly, GLMM were fitted to test the effect of single candidate indicator variables on each response measure, where $p< 0.01$ the effect and direction of the relationship was examined. For each single variable model, cow was included as a random effect with each candidate indicator being included as a fixed effect. Multi-variable models were then constructed for each response measure. Variables which had shown to be highly correlated to one another were not entered into the same model. P-values and model coefficients for each model were produced.

(iv) Assessing model performance

Goodness of fit was assessed by examining the sensitivity (proportion of true positives to predicted positives) and specificity (proportion of true negatives to predicted negatives). Sensitivity and specificity are common ‘clinimetric’ parameters that define the ability of a measure to detect the presence or absence of a specific condition and although they must be considered together, they trade off with each other (Arezzo *et al*., 2013). In the context of human epidemiology, a highly sensitive test is one that is good at including most people who have a condition whereas a highly specific test is one that is good at excluding most people who do not have a condition (Borst, 2020). Both sensitivity and specificity are influenced by the complexity of the condition being studied, the prevalence of the condition in the sampled population and population homogeneity i.e. how similar the members of the study population are to one another (Arezzo *et al*., 2013). The sensitivity and specificity were calculated by varying the threshold for the estimated probability above which the GLMM judges there to be a health problem and comparing the estimates of whether there is a health problem for each cow-lactation with the true data; testing at various thresholds is essential when dealing with binary responses.

Receiver Operating Characteristic (ROC) curves, a well-known means for evaluating diagnostic tools in medicine, were used to further assess goodness of fit (Grimnes & Martinsen, 2015). The ROC curve is a graphical tool which is useful for visualising the performance of a binary (having two possible outcomes) classifier and thus can be used to investigate the discriminatory power of a model (Tasche, 2008; Grimnes & Martinsen, 2015). There are four possible outcomes from a binary classification process (Trucco *et al*., 2019):

(1) data which are correctly identified as positive (true positive) e.g. a cow with mastitis being classified as having disease by the model
(2) data which are incorrectly classified as negative (false negative) e.g. a cow with mastitis being classified as not having mastitis

(3) data which are correctly identified as negative (true negative) e.g. a cow which does not have mastitis being classified as not having mastitis
(4) data which are incorrectly classified as positive (false positive) e.g. a cow which does not have mastitis being classified as having mastitis.

Thus, an ROC curve visualises the diagnostic ability of a model to correctly identify the presence or absence of a condition by plotting the true positive rate (on the y-axis) against the false positive rate (on the x-axis) and generating a curve in which each point represents a sensitivity-specificity pair corresponding to a particular classification threshold (Trucco *et al.*, 2019). At a probability threshold of 0.5, observations with a probability greater than or equal to 0.5 are classified as having the condition (Yes) and those observations with a probability of less than 0.5 are classified as not having the condition (No). In most real cases, the distributions of ‘Yes’ and ‘No’ populations will overlap and thus varying the classification threshold allows manipulation of the sensitivity and specificity to optimise model performance (Grimnes & Martinsen, 2015). Plotting ROC curves provides information about the overlap between two classes (Yes and No); in many cases the mean values of these classes may differ significantly, yet their variance is so large that correct class distinction is impeded (Meyer-Baese & Schmid, 2014). The ROC curve of a good classifier is closer to the top left of the plot (solid line in Figure 4.3), which indicates the model is achieving a high true positive rate concurrent with a low false positive rate. A poor classifier is less able to distinguish between true positives and false positives and produces an ROC curve close to diagonal (dashed line in Figure 4.3).

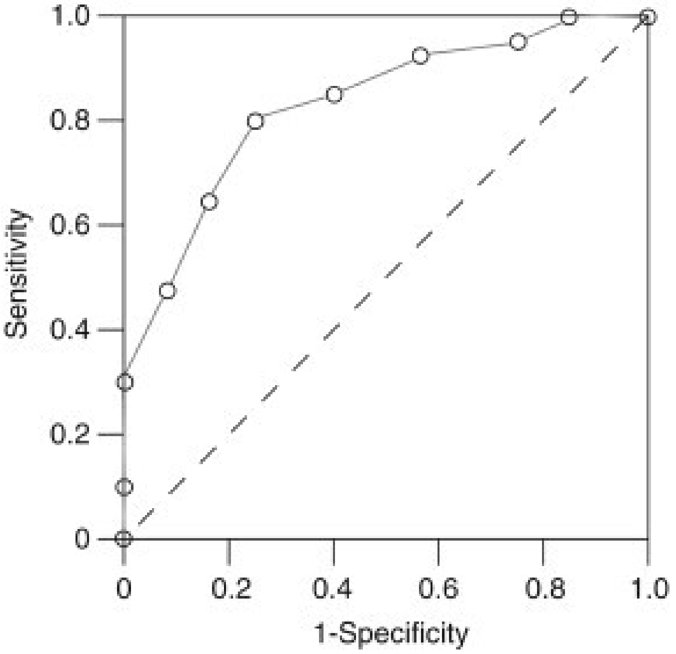


Figure 4:3: Example of a basic ROC curve (Grimnes & Martinsen, 2015) Reproduced with permission.

4.4 Results

4.4.1 Exploring distributions

Distributions were visually examined for each variable and factor according to health outcome. Figure 4.4 provides an example of the distributions of condition score at dry off for cows with no reproductive disease in the first 30 days of lactation and cows with reproductive disease in the first 30 days of lactation.

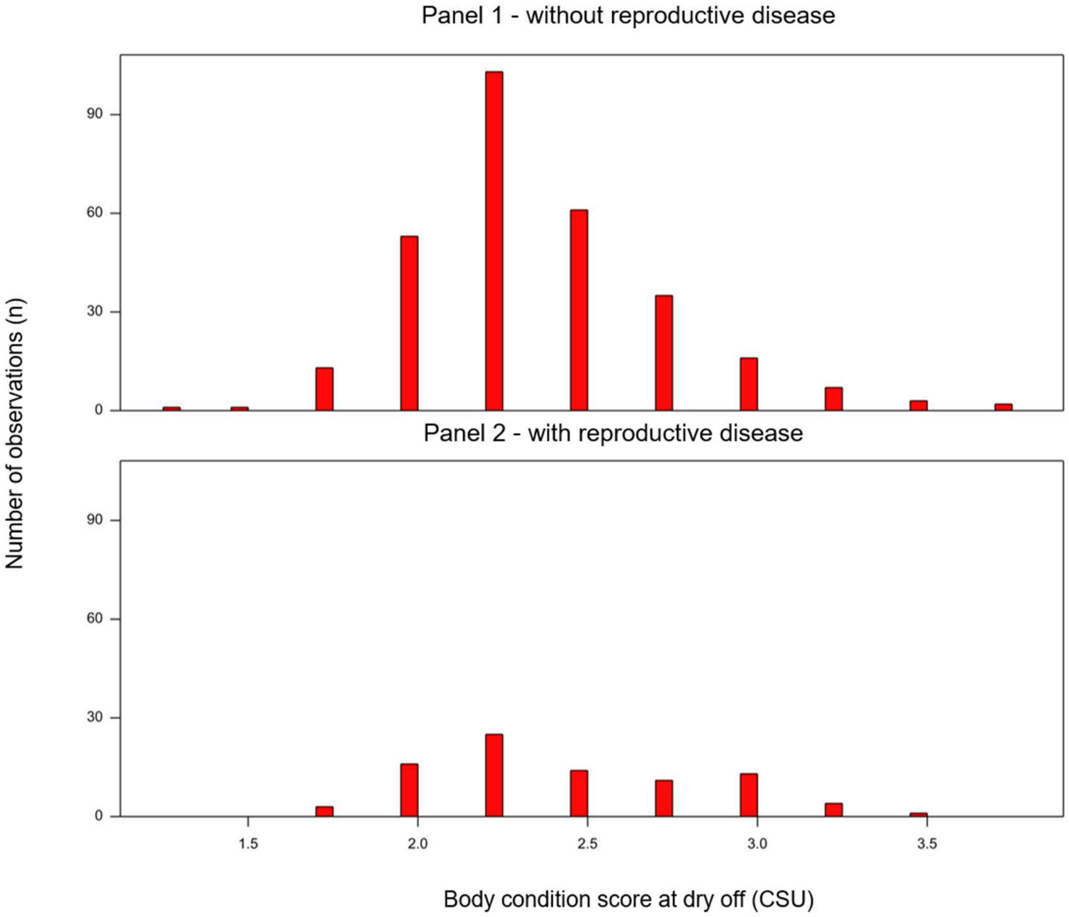


Figure 4.4: Histogram of condition score at dry off for cow-lactations without reproductive disease (Panel 1) and with reproductive disease (Panel 2)

Figure 4.4 shows that there is very little discrimination in condition score at dry off between cows that did not develop reproductive disorders and those that did, despite significantly different means between the populations. Figure 4.5 provides a further example of the distribution of milk yield at dry off for the population of cows without a mastitis diagnosis in the first 30 days of the following lactation relative to the distribution within the population of cows that did have mastitis. A similar pattern is seen – despite significant differences in the mean yield between the two populations, the range of yields in both populations means there is limited discriminatory power in terms of yield at dry off.

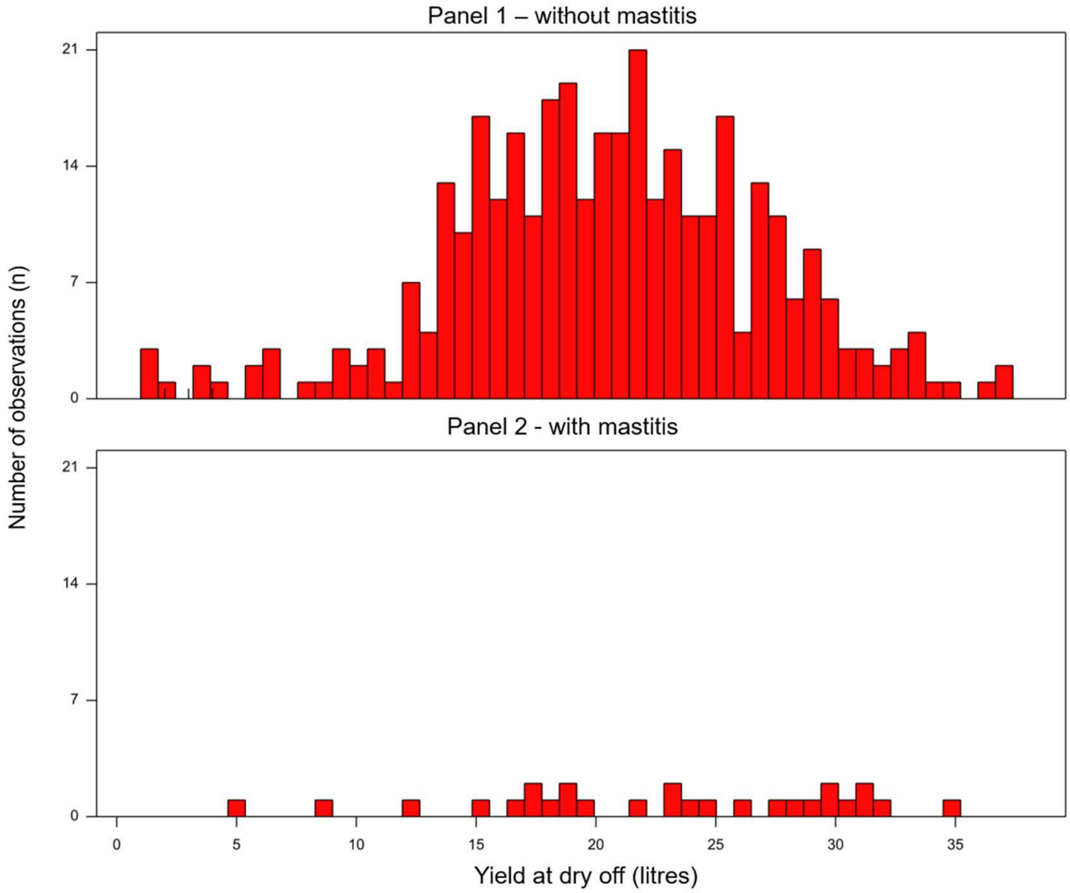


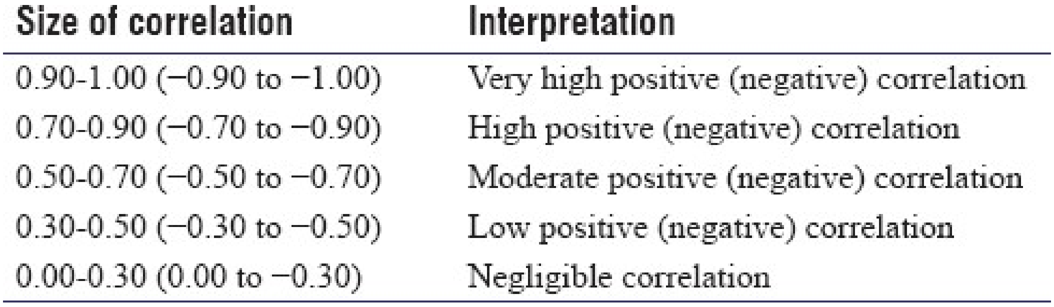
Figure 4.5: Histogram of milk yield at dry off for cow-lactations without mastitis (Panel 1) and with mastitis (Panel 2)

These examples are representative of all the distribution plots drawn for each candidate indicator relative to each health outcome which all demonstrated large overlapping ranges of observations between groups (with vs. without disease).

4.4.2 Understanding interrelationships

Significant correlations were found to exist between many of the candidate variables (Table 4.3). All the candidate variables (measures and estimates) associated with liveweight between drying and calving are highly correlated (Pearson's ρ>0.76). Measurements and estimates from regression of body weight between dry off and calving are highly correlated with estimates from regression of body energy content (Pearson’s ρ>0.85) and to a lesser with body energy content measurements at dry off and calving (Pearson’s ρ>0.46). This high level of correlation has important implications for variable selection for the development of the GLMM models. Table 4.2 provides a key for interpretation of the coefficients presented in Table 4.3.

Table 4.2: Interpretation of correlation coefficients taken from Hinkle *et al.*, 2003. Reproduced with permission.



Size of correlation	Interpretation
0.90-1.00 (−0.90 to −1.00)	Very high positive (negative) correlation
0.70-0.90 (−0.70 to −0.90)	High positive (negative) correlation
0.50-0.70 (−0.50 to −0.70)	Moderate positive (negative) correlation
0.30-0.50 (−0.30 to −0.50)	Low positive (negative) correlation
0.00-0.30 (0.00 to −0.30)	Negligible correlation

Table 4.3: Pearson correlation matrix for candidate explanatory variables with colour scale indicating strength of Pearson's ρ and where p< 0.05 (see Table 4.2 for key)

	Dry period length	BW at drying	BW at calving	BW change	BW intercept (calving)	BW intercept (-60)	BW slope	CS at drying	CS at calving	CS change	CS intercept (calving)	CS intercept (-60)	CS slope	BEC at drying	BEC at calving	BEC change	BEC intercept (calving)	BEC intercept (-60)	BEC slope	Yield at drying
Dry period length	1.000																			
BW at drying	-0.018	1.000																		
BW at calving	0.078	0.758	1.000																	
BW change	0.276	-0.433	0.218	1.000																
BW intercept (calving)	0.098	0.839	0.863	-0.070	1.000															
BW intercept (-60)	0.126	0.878	0.776	-0.221	0.847	1.000														
BW slope	-0.062	-0.133	0.075	0.286	0.195	-0.356	1.000													
CS at drying	0.007	0.537	0.401	-0.241	0.452	0.501	-0.130	1.000												
CS at calving	0.042	0.375	0.429	0.016	0.401	0.398	-0.030	0.603	1.000											
CS change	0.137	-0.316	-0.075	0.412	-0.166	-0.226	0.126	-0.642	0.181	1.000										
CS intercept (calving)	0.032	0.465	0.423	-0.131	0.466	0.466	-0.040	0.658	0.768	-0.105	1.000									
CS intercept (-60)	0.053	0.548	0.413	-0.246	0.443	0.549	-0.231	0.806	0.598	-0.411	0.626	1.000								
CS slope	-0.035	-0.241	-0.122	0.187	-0.104	-0.236	0.250	-0.377	-0.008	0.416	0.182	-0.653	1.000							
BEC at drying	0.072	0.693	0.478	-0.335	0.576	0.595	-0.081	0.657	0.410	-0.431	0.480	0.587	-0.276	1.000						
BEC at calving	0.123	0.455	0.587	0.028	0.489	0.480	-0.030	0.409	0.566	0.012	0.474	0.435	-0.094	0.651	1.000					
BEC change	0.128	-0.429	-0.021	0.740	-0.194	-0.218	0.062	-0.399	0.051	0.592	-0.143	-0.288	0.228	-0.602	0.185	1.000				
BEC intercept (calving)	0.090	0.849	0.862	-0.118	0.995	0.849	0.190	0.450	0.414	-0.157	0.482	0.462	-0.111	0.590	0.496	-0.212	1.000			
BEC intercept (-60)	0.123	0.887	0.780	-0.256	0.854	0.990	-0.321	0.501	0.404	-0.222	0.462	0.560	-0.253	0.613	0.498	-0.222	0.847	1.000		
BEC slope	-0.065	-0.115	0.080	0.261	0.192	-0.316	0.927	-0.125	-0.009	0.129	0.005	-0.210	0.268	-0.077	-0.041	0.033	0.213	-0.339	1.000	
Yield at drying	-0.130	-0.164	-0.072	0.160	-0.098	-0.172	0.142	-0.319	-0.217	0.178	-0.208	-0.366	0.262	-0.318	-0.204	0.250	-0.109	-0.201	0.173	1.000

4.4.3 Single variable models

(i) Reproductive Disorders – GLMM & assessing model performance

All GLMM models for reproductive disorders ran successfully. Twelve candidate indicators were found to be significant for reproductive disease when included singly as fixed effects in the model; dry period length (p= 0.01), BW at dry off (p= 0.007), BW change (p= 0.000), BW intercept 60 days before calving (p= 0.036), CS at dry off (p= 0.008), CS change (p= 0.003), CS intercept 60 days before calving (p= 0.009), CS slope (p= 0.019), BEC at dry off (p=0.009), BEC change (p< 0.001), BEC intercept at calving (p= 0.045) and BEC intercept at 60 days before calving (p = 0.014) (Table 4.4). Candidate indicators taken as single time point measures all had large positive effects whereas those calculated as rates of change over time were negative. However, the individual cow variance components and their associated very large standard errors show that a large proportion of the variation between cows is not explained by the variables used in these models.

With regards to dry period length, Figure 4.6 shows the mean predicted probability, and associated standard error, of a cow having a reproductive disease at 20 equidistant points between the maximum and minimum observed values for deviation from dry period length, in days. On the transformed scale, the proportion of cows with a reproductive disorder increases from 15% for cows with a mean dry period length to over 70% for cows with a dry period length, 10 days shorter or longer than mean dry period length.

Despite the large and statistically significant effect seen in Figure 4.6, the fit of the model was poor due to the large overlap in the distributions of observed dry period lengths between cow-lactations with reproductive disorders and those without reproductive disorders, as demonstrated in the poor specificity and sensitivity of dry period length as a predictor for reproductive disease in early lactation (Figures 4.7). The poor predictive nature of dry period length is further illustrated by the ROC curve (Figure 4.8) which is close to diagonal, meaning that the model is unable to distinguish between true positives and false positives with sufficient accuracy - see Figure 4.3 for example curves for good and poor predictors. The near-diagonal ROC curve reflects that the performance of dry period length as a diagnostic test is no better than chance i.e. the positive and negative results are not related to true disease status.

Table 4.4: Individual cow variance components with associated standard error and test statistics from GLMMs for reproductive disorders with each variable included as a fixed effect and cow as a random effect

Variable type	Variable	Individual cow variance component	Standard error of variance component	Wald Statistic	Numerator degrees of freedom	p-value (Wald)	F-statistic	Denominator degrees of freedom	p-value (F-statistic)	p-value	Direction of effect for p<0.05
Factor	Cycle number	0.31064	0.36363	0.437	1	0.508				0.508	
Factor	Month of dry off	0.26907	0.36834	11.433	11	0.408				0.408	
Factor	Month of calving	0.30570	0.36952	10.655	11	0.473				0.473	
Factor	Genetic line	0.28331	0.36413	2.061	1	0.151	2.061	346	0.152	0.152	
Factor	Diet type	0.32061	0.36446	0.011	1	0.915	0.011	348	0.915	0.915	
Covariate	Year of dry off	0.31919	0.36434	0.071	1	0.790	0.071	479	0.791	0.791	
Covariate	Year of calving	0.31720	0.36425	0.357	1	0.550	0.357	469	0.551	0.551	
Covariate	Dry period length	0.34650	0.37020	6.660	1	0.010	6.660	425	0.010	0.010*	Positive
Covariate	BW at dry off	0.21852	0.37505	7.440	1	0.006	7.440	436	0.007	0.007**	Negative
Covariate	BW at calving	0.32616	0.36615	0.521	1	0.471				0.471	
Covariate	BW change	0.23715	0.38774	12.482	1	0.000	12.482	397	0.000	0.000***	Negative
Covariate	BW intercept (calving)	0.13471	0.38108	2.854	1	0.091				0.091	
Covariate	BW intercept (-60)	0.14572	0.38098	4.409	1	0.036				0.036**	Positive
Covariate	CS at dry off	0.26372	0.36200	7.048	1	0.008	7.048	460	0.008	0.008**	Positive
Covariate	CS change	0.25990	0.36435	8.845	1	0.003	8.845	489	0.003	0.003**	Negative
Covariate	CS intercept (-60)	0.29906	0.37520	6.967	1	0.008	6.967	463	0.009	0.009**	Positive
Covariate	CS slope	0.33742	0.37797	5.519	1	0.019				0.019*	Negative
Covariate	BEC at dry off	0.25350	0.37834	6.845	1	0.009	6.845	473	0.009	0.009**	Positive
Covariate	BEC at calving	0.33213	0.36681	0.804	1	0.370				0.370	
Covariate	BEC change	0.23885	0.38598	14.059	1	0.000	14.059	375	0.000	0.000***	Negative
Covariate	BEC intercept (calving)	0.21499	0.39763	4.035	1	0.045	4.035	446	0.045	0.045*	Positive
Covariate	BEC intercept (-60)	0.21244	0.39766	6.031	1	0.014				0.014**	Positive
Covariate	BEC slope	0.28183	0.39975	0.933	1	0.334				0.334	
Covariate	Yield at dry off	0.00152	0.42103	2.576	1	0.108	2.576	331	0.109	0.109	

* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001

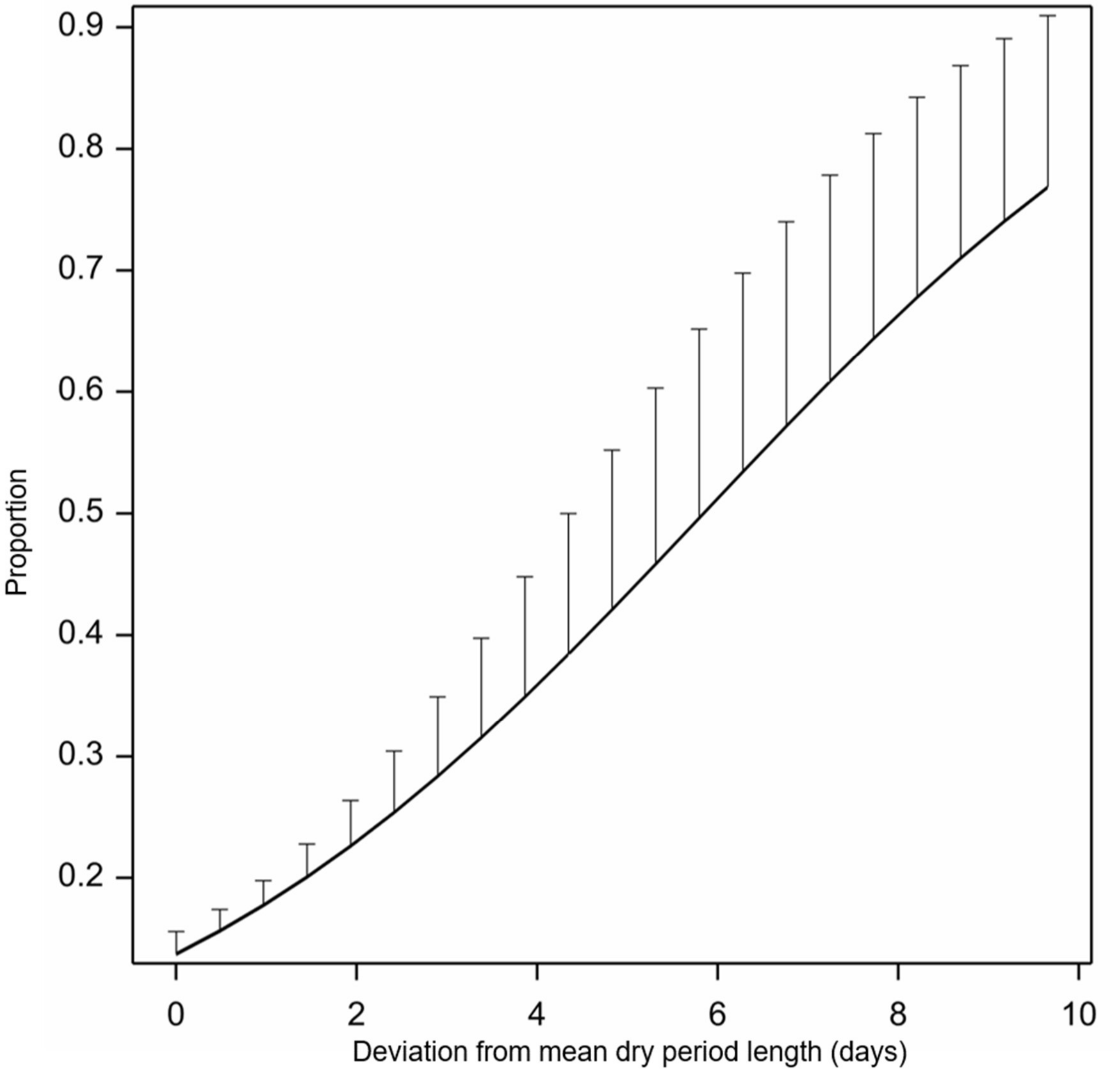


Figure 4.6: Proportion of cow-lactations with reproductive disorders estimated from GLMM with dry period length included as a fixed effect shown as a function of deviation from mean dry period length (days)

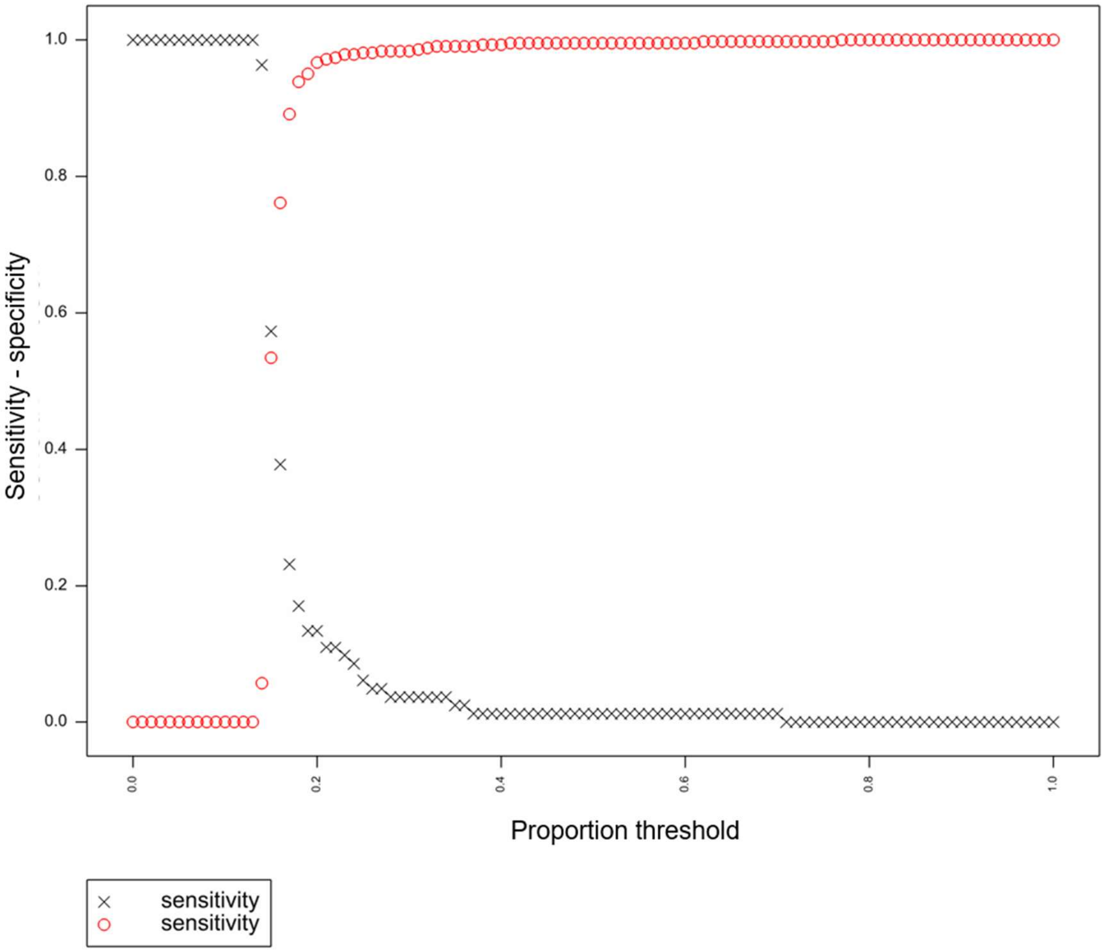


Figure 4.7: Sensitivity and specificity based on proportion threshold of cow-lactations with reproductive disorders in early lactation estimated from GLMM with dry period length included as a fixed effect

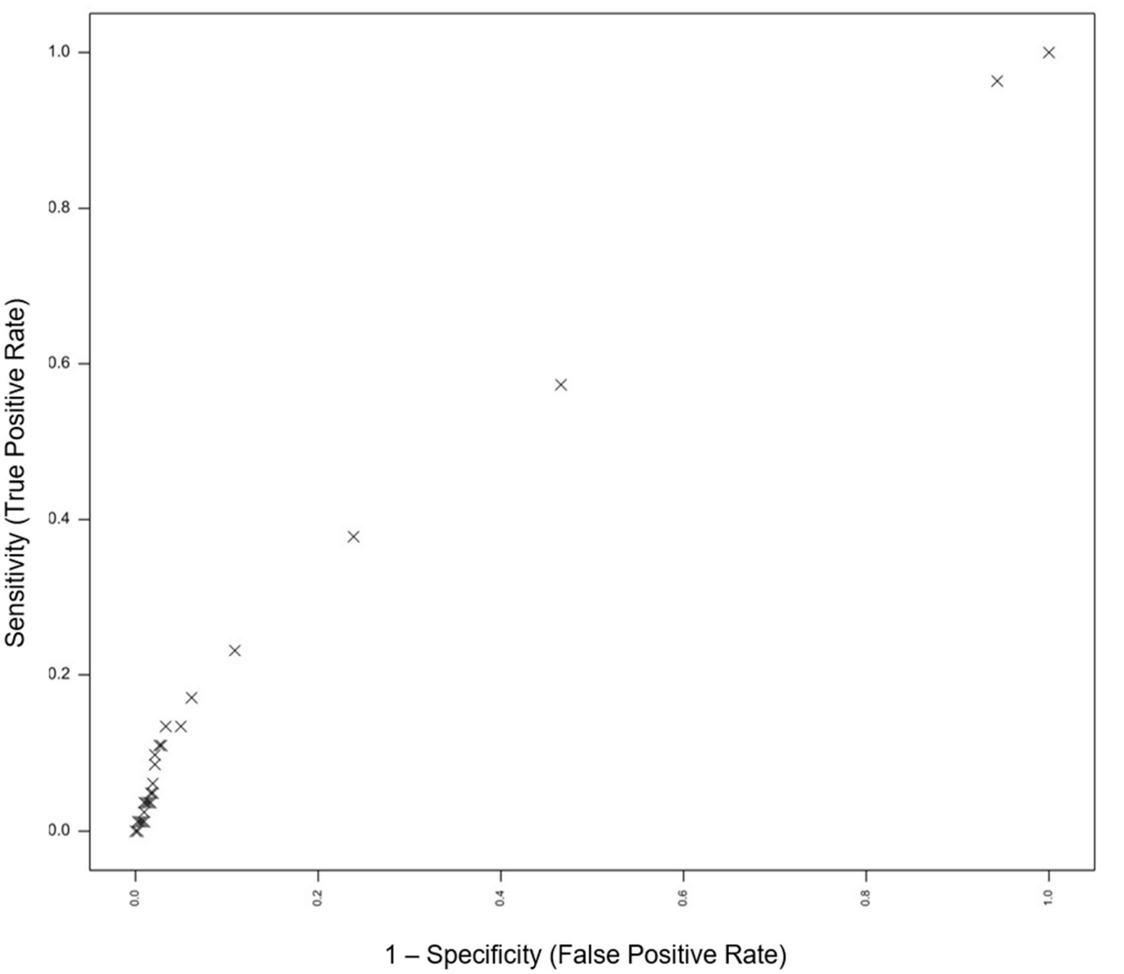


Figure 4.8: ROC Curve based on proportion threshold of cow-lactations with reproductive disorders in early lactation estimated from GLMM with dry period length included as a fixed effect (Optimum sensitivity = 0.31, optimum specificity = 0.82)

Body weight at dry off was also found to be significant as a predictor of reproductive disorders (p=0.007) (Table 4.4). The nature of the effect means that incidence of reproductive disorders increases with increasing body weight at dry off. The risk of reproductive disorders increases from less than 10% for cows weighing 500kg at dry off to over 35% for cows weighing 900kg at dry off (Figure 4.9). Despite the large and statistically significant effect seen in Figure 4.9, the fit of the model was poor due to the large overlap in the distributions between cow- lactations with and those without metabolic disorders as plotted in Figure 4.10. This histogram illustrates that despite a significant difference in mean liveweight at dry off between the 2 populations (with and without disease), the range of observations within each population are similar and thus preclude it as an accurate predictive measure. Consequently, the specificity and sensitivity (which are inversely proportional to one another) of body weight change as a predictor for reproductive disease in early lactation were poor (Figure 4.11), as further illustrated by the ROC curve which is close to diagonal (Figure 4.12). When specificity is optimised so that 79% of true negatives are identified (i.e. a cow without disease is predicted to have no disease), only 31% of true positives were correctly identified.

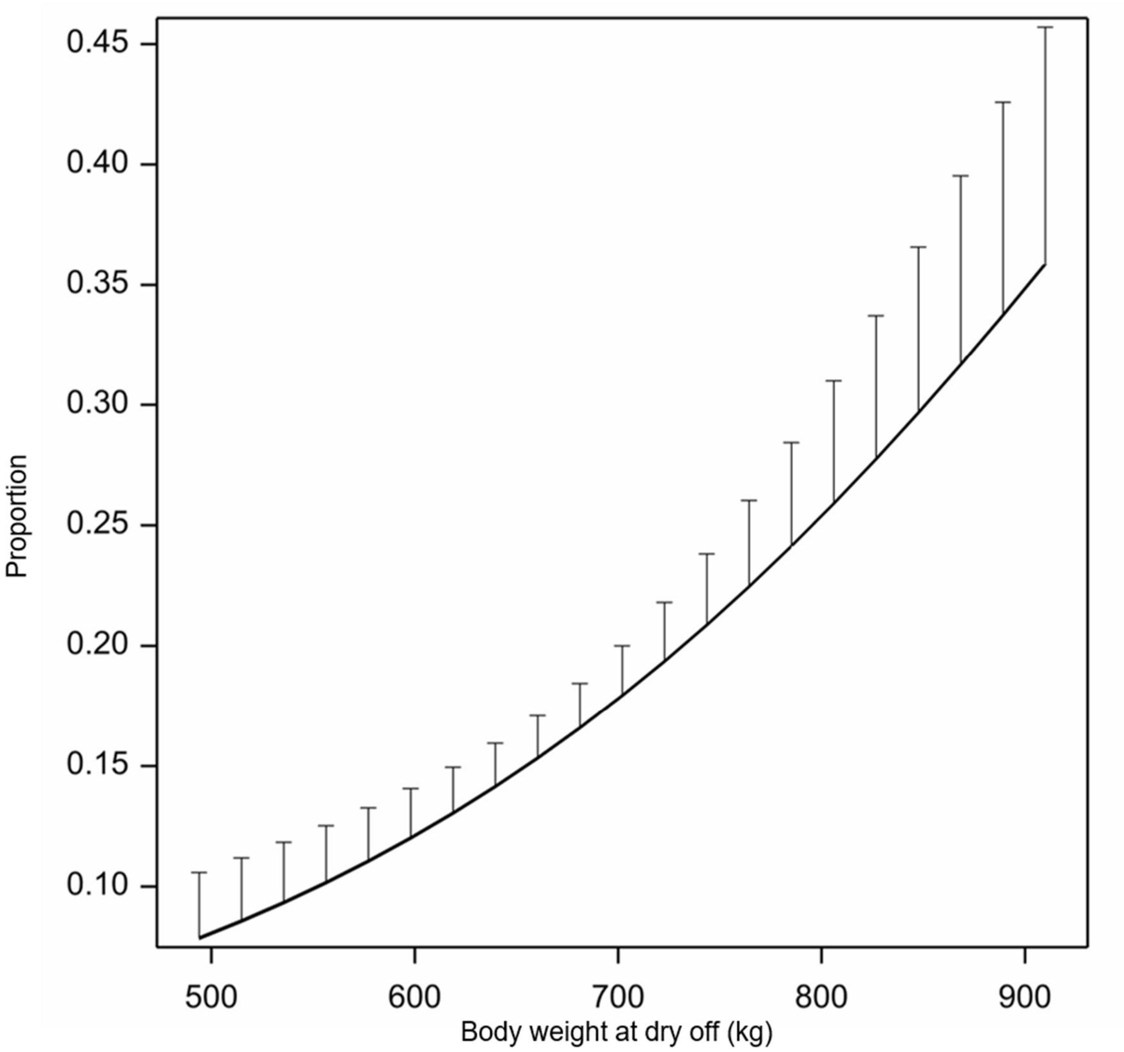


Figure 4.9: Proportion of cow-lactations with reproductive disorders estimated from GLMM with body weight at dry off included as a fixed effect shown as a function of body weight at dry off (kg)

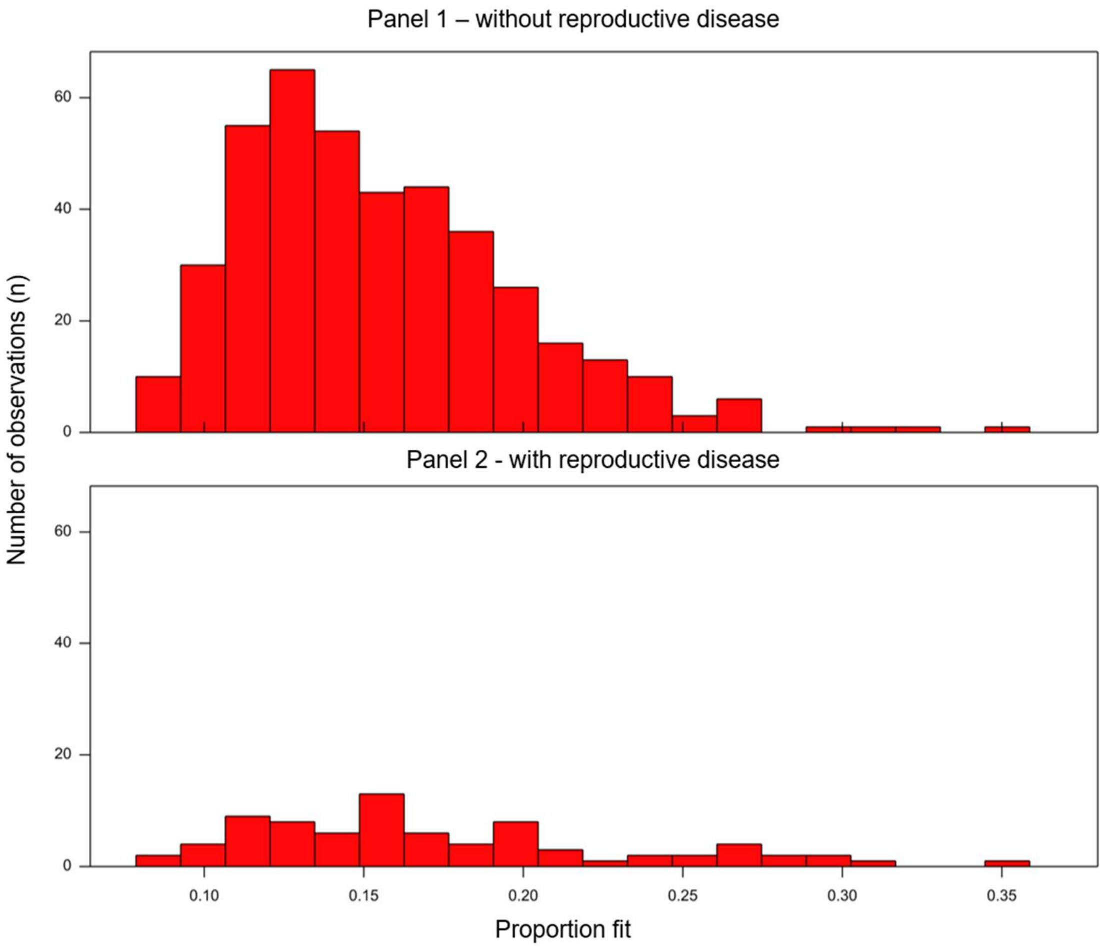


Figure 4.10: Histograms of proportion fit of cow-lactations with reproductive disorders estimated from GLMM with body weight at dry off included as a fixed effect for cow-lactations without (Panel 1) and with reproductive disorders (Panel 2)

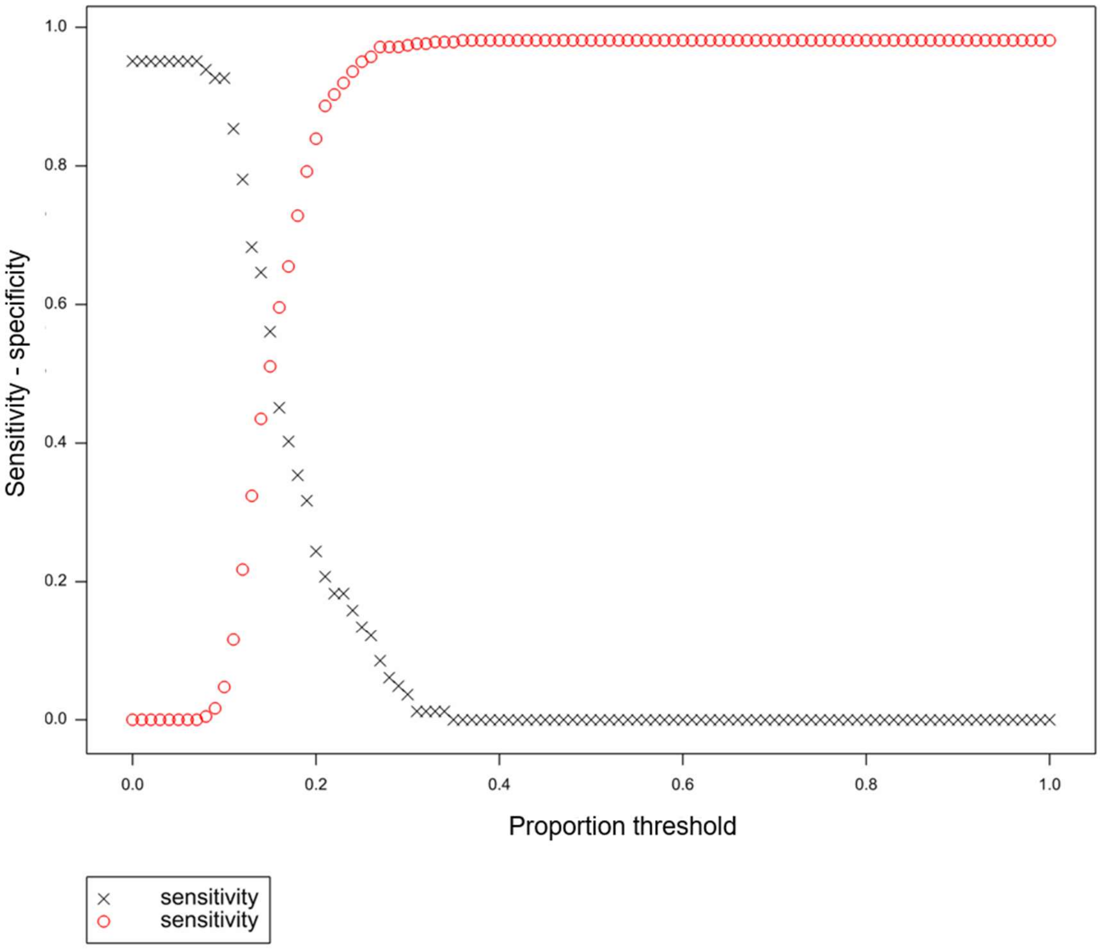


Figure 4.11: Sensitivity and specificity based on thresholds of proportion of cow-lactations with reproductive disorders in early lactation estimated from GLMM with body weight at dry off included as a fixed effect

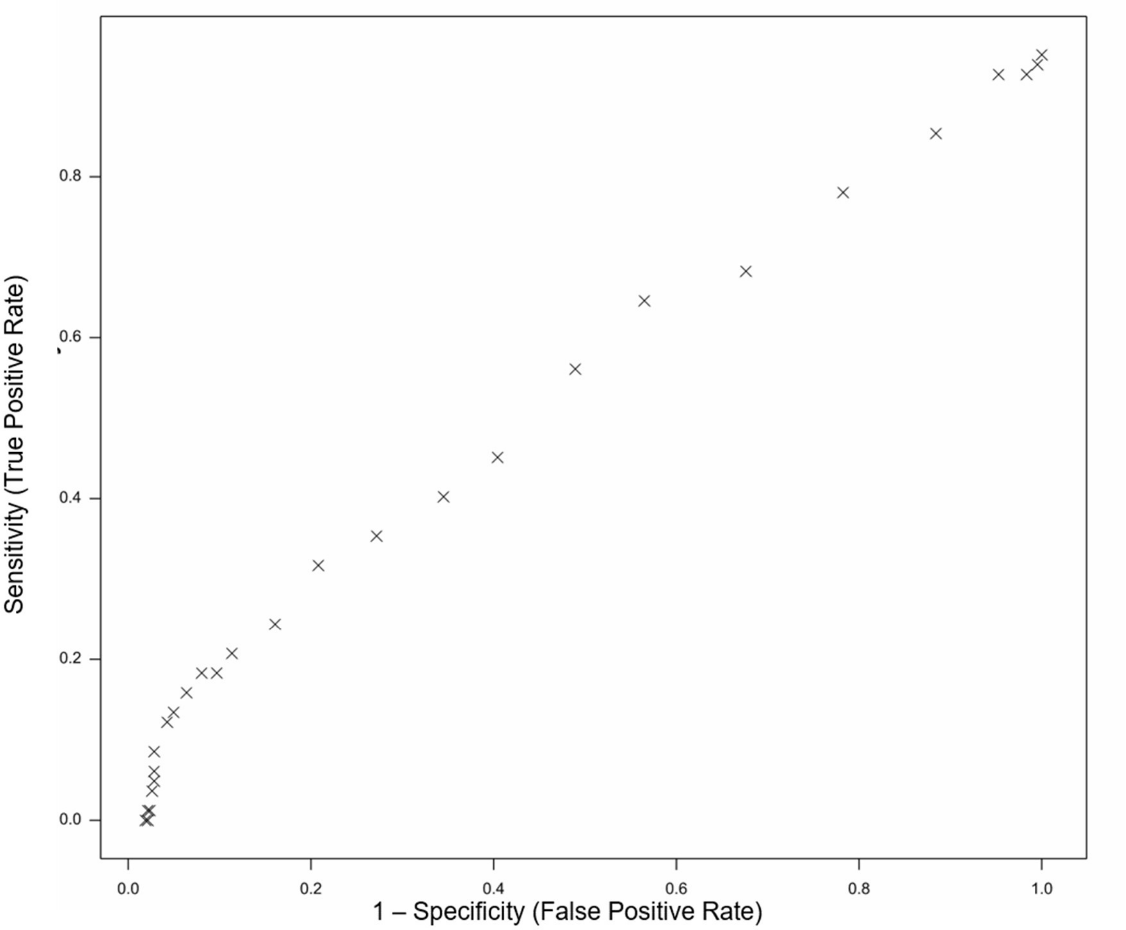


Figure 4.12: ROC Curve based on thresholds of proportions of cow-lactations with reproductive disorders in early lactation estimated from GLMM with body weight at dry off included as a fixed effect (Optimum sensitivity = 0.31, optimum specificity = 0.79)

In summary, despite 12 candidate indicators being found to be significant ($p< 0.05$) for reproductive disease; the populations (with disease and without disease) had similar distributions and ranges of observed values for each of the candidate indicators as seen in the example histograms. Therefore, the ability of each of these candidate indicators to be used successfully in a binary classification system is limited, even when the proportion threshold is varied. Thus, these candidate indicators cannot be said to be able to distinguish between true positive and false positive cases and consequently produced ROC curves close to diagonal.

(ii) Metabolic disorders – GLMM & assessing model performance

Due to sparse data, two of the GLMM models (month of calving and month of drying) failed. Five of the candidate variables were found to be significant predictors for metabolic disease (Table 4.5). Cycle number, which relates to parity, was the only factor found to be significant; cows moving to lactation 3 had an increased risk of metabolic disease compared to cows moving to lactation 2. Body weight at drying and the intercepts of body weight at calving, at 60 days before calving and body energy content at calving all had significant positive effects on risk of metabolic disorder incidence.

The intercept of body energy content at calving, calculated from the logistic regression, was significant ($p = 0.034$) for metabolic disorders. The large positive nature of the relationship means that the increase in risk for metabolic disorder increases from less than 1% to 15% across the range of body energy content intercepts at calving observed in the current study, although the associated standard errors are very large (Figure 4.13).

Despite the large and statistically significant effect seen in Figure 4.13, the fit of the model was poor as there was no discrimination between populations according to the intercept of body energy content at calving, although relative to sample size, the proportion of cows at the higher end of the distribution appears to be greater in cows with metabolic conditions (Figure 4.14).

Overall, the specificity and sensitivity of body energy content (intercept at calving) as a predictor for metabolic disease in early lactation was poor (Figure 4.15 & Figure 4.16) with the model failing to accurately distinguish between cows which had metabolic disease and those which did not. At the optimum specificity achieved by the model (0.69), it was only able to correctly identify 30% of cows with disease.

Table 4.5: Individual cow variance components with associated standard error and test statistics from GLMMs for metabolic disorders with each variable included as a fixed effect and cow as a random effect

Variable type	Variable	Individual cow variance component	Standard error of variance component	Wald Statistic	Numerator degrees of freedom	p-value (Wald)	F-statistic	Denominator degrees of freedom	p-value (F-stat)	P-value	Direction of effect for p <0.05
Factor	Cycle number	0.77036	0.95032	6.750	1	0.000				0.009*	3>2
Factor	Month of dry off				GLMM failed due to sparse data						
Factor	Month of calving				GLMM failed due to sparse data						
Factor	Genetic line	0.66653	0.95935	2.671	1	0.102				0.102	
Factor	Diet type	0.66646	0.95946	0.062	1	0.803	0.062	484.6	0.803	0.803	
Covariate	Year of dry off	0.70332	0.95828	0.647	1	0.421				0.421	
Covariate	Year of calving	0.69731	0.95623	0.874	1	0.350				0.350	
Covariate	Dry period length	0.65543	0.95903	0.004	1	0.949				0.949	
Covariate	BW at dry off	0.78669	0.93197	5.772	1	0.016				0.016*	Positive
Covariate	BW at calving	0.70159	0.98922	2.972	1	0.085				0.085	
Covariate	BW change	0.74223	1.00540	0.241	1	0.623				0.623	
Covariate	BW intercept (calving)	0.71276	0.91780	7.177	1	0.007				0.007*	Positive
Covariate	BW intercept (-60)	0.78466	0.92975	5.206	1	0.023				0.023*	Positive
Covariate	CS at dry off	0.66042	0.95906	0.001	1	0.975				0.975	
Covariate	CS change	0.70118	0.95344	1.244	1	0.265				0.265	
Covariate	CS intercept (-60)	0.64201	0.97811	0.273	1	0.601				0.601	
Covariate	CS slope	0.66658	0.97875	0.019	1	0.892				0.892	
Covariate	BEC at dry off	0.69930	0.95483	1.645	1	0.200				0.200	
Covariate	BEC at calving	0.78766	1.00375	0.169	1	0.681				0.681	
Covariate	BEC change	0.76210	1.00120	0.398	1	0.528				0.528	
Covariate	BEC intercept (calving)	0.78505	0.94551	4.480	1	0.034				0.034*	Positive
Covariate	BEC intercept (-60)	0.83491	0.95455	3.009	1	0.083				0.083	
Covariate	BEC slope	0.70467	0.96847	0.354	1	0.552				0.552	
Covariate	Yield at dry off	0.92535	1.09883	1.160	1	0.281				0.281	

* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001

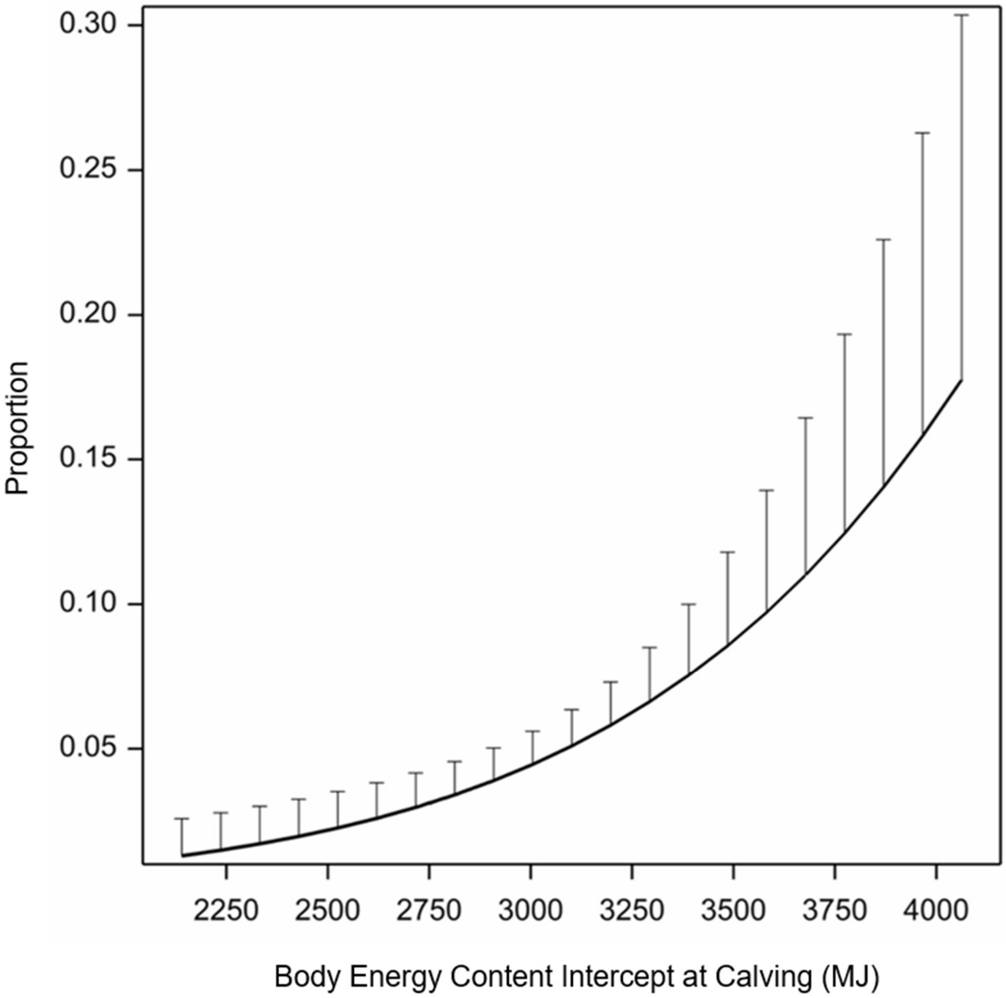


Figure 4.13: Proportion of cow-lactations with metabolic disorders estimated from GLMM with body energy content (intercept at calving) included as a fixed effect shown as a function of body energy (intercept at calving)

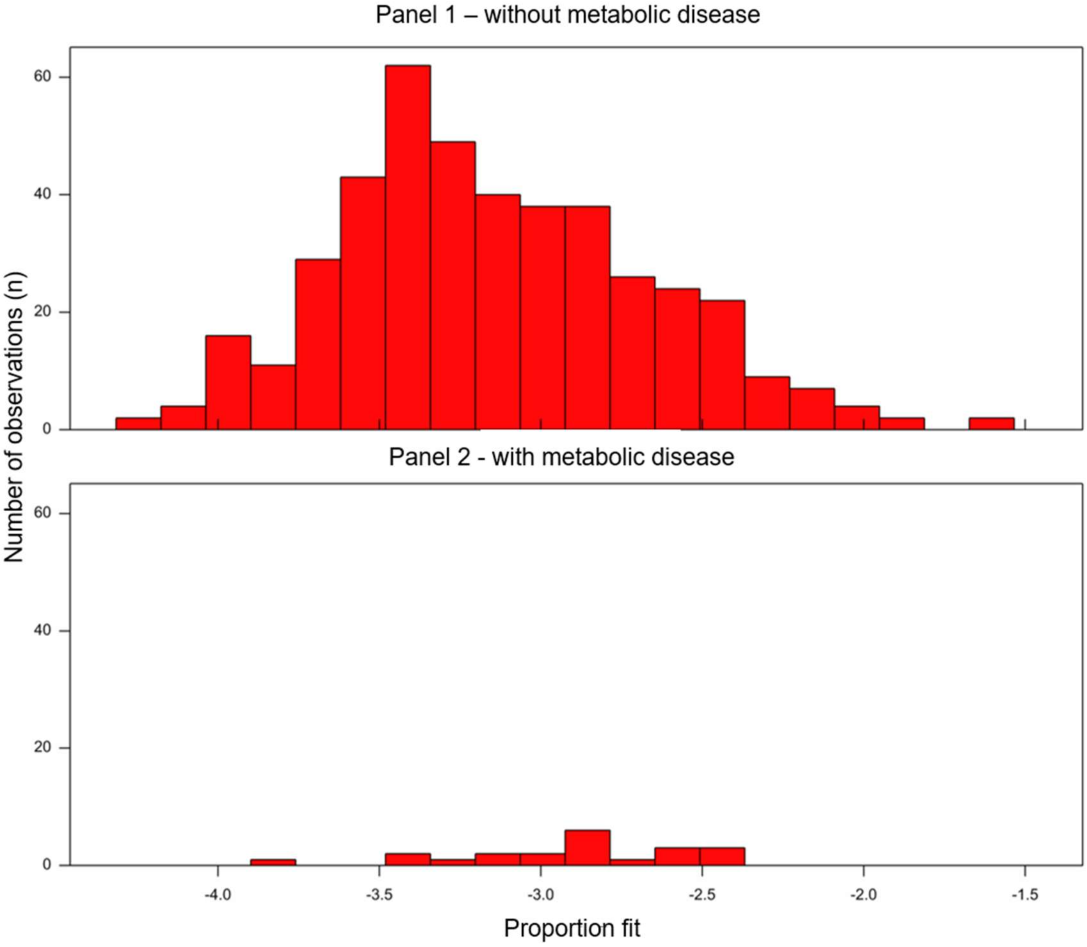


Figure 4.14: Histograms of proportion of cow-lactations with metabolic disorders estimated from GLMM with body energy content (intercept at calving) included as a fixed effect for cow-lactations without (Panel 1) and without (Panel 2) metabolic disorders

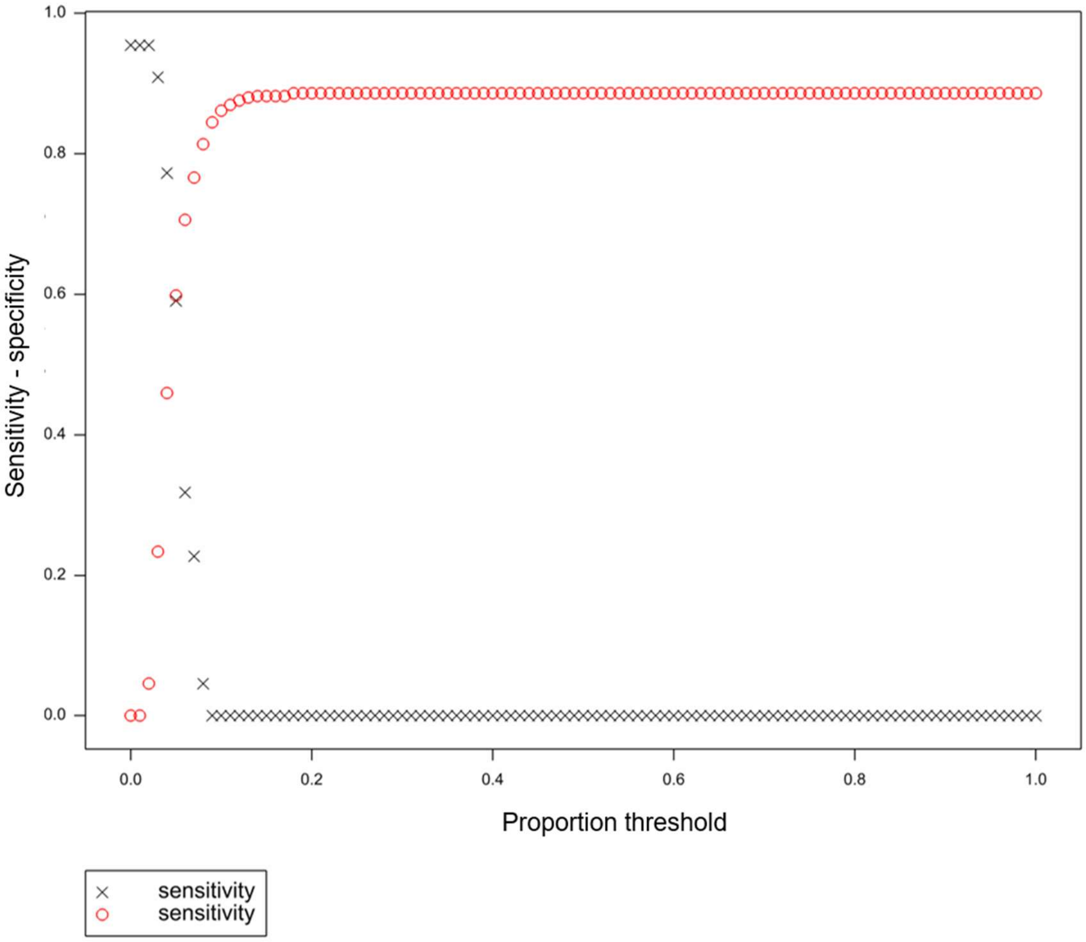


Figure 4.15: Sensitivity and specificity based on thresholds of proportion of cow-lactations with metabolic disorders in early lactation estimated from GLMM with body energy content (intercept at calving) included as a fixed effect

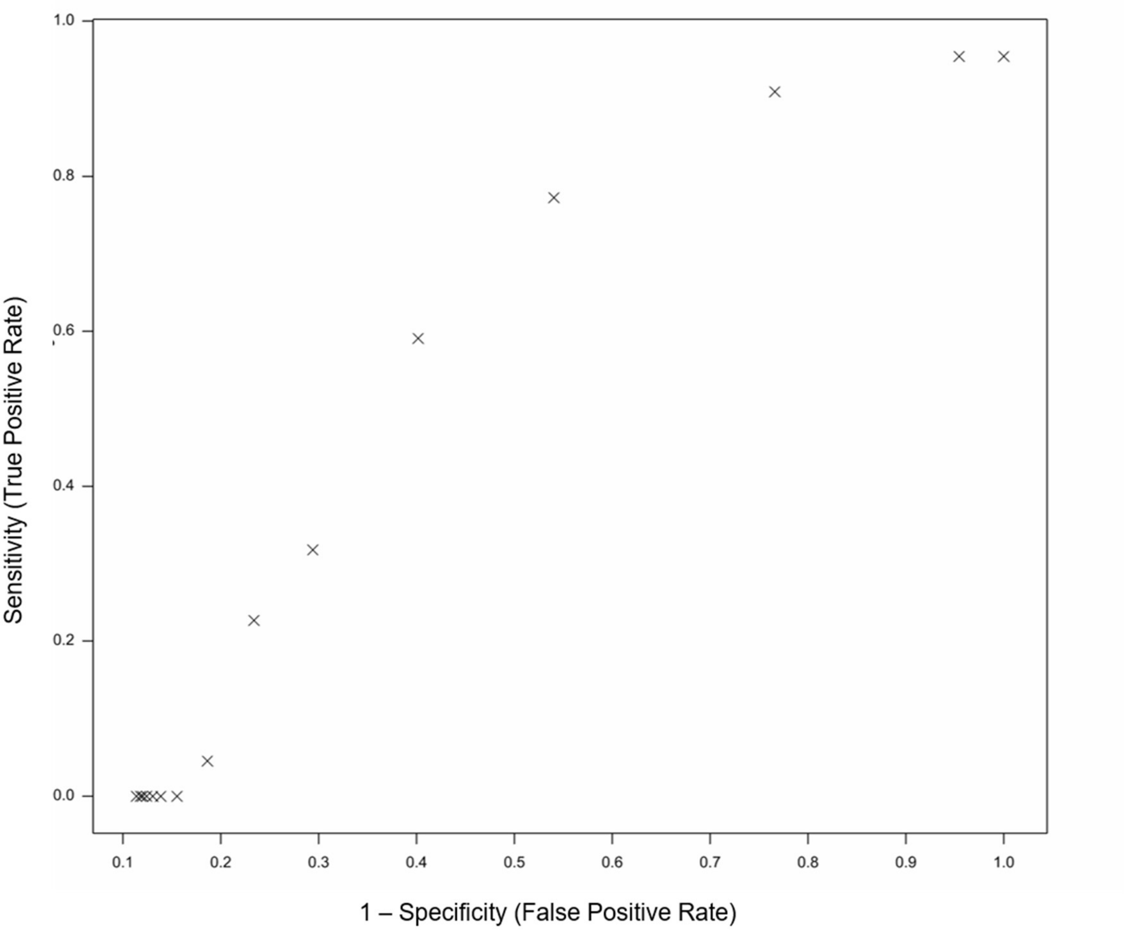


Figure 4.16: ROC Curve based on thresholds of proportions of cow-lactations with metabolic disorders in early lactation estimated from GLMM with body energy content (intercept at calving) included as a fixed effect (Optimum sensitivity = 0.30, optimum specificity = 0.69)

Body weight at dry off was also found to be a significant factor for metabolic disease (p= 0.016). The nature of the large positive effect means that as body weight at dry off increases so too does the incidence of metabolic disease. The incidence of metabolic disease increased, from below 5% to over 15% as body weight at dry off increased from 500 to 900kg (Figure 4.17). The range of observed values of body weight at dry off for the two populations (with or without metabolic disease) was similar and therefore the discriminatory power of this candidate indicator is limited (Figure 4.18, 4.19 & 4.20).

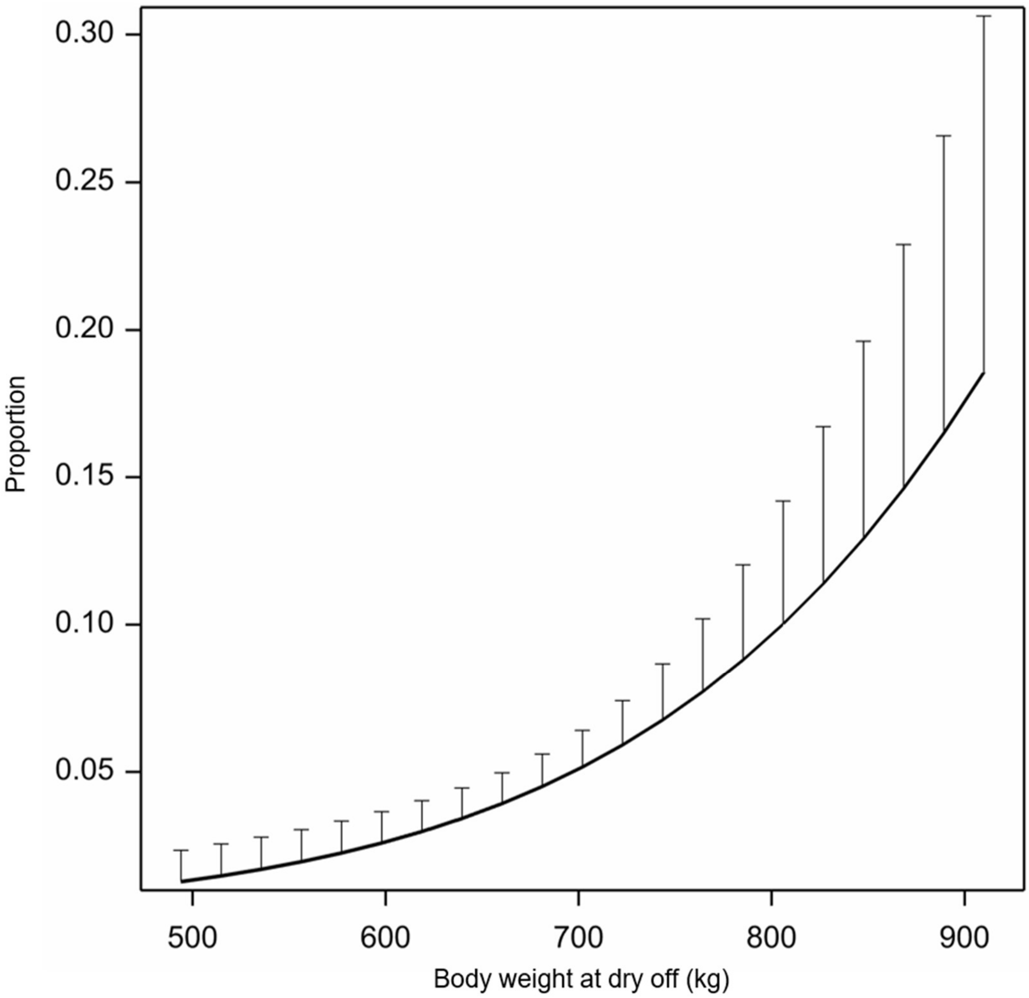


Figure 4.17: Proportion of cow-lactations with metabolic disorders estimated from GLMM with body weight at dry off included as a fixed effect shown as a function of body weight at dry off

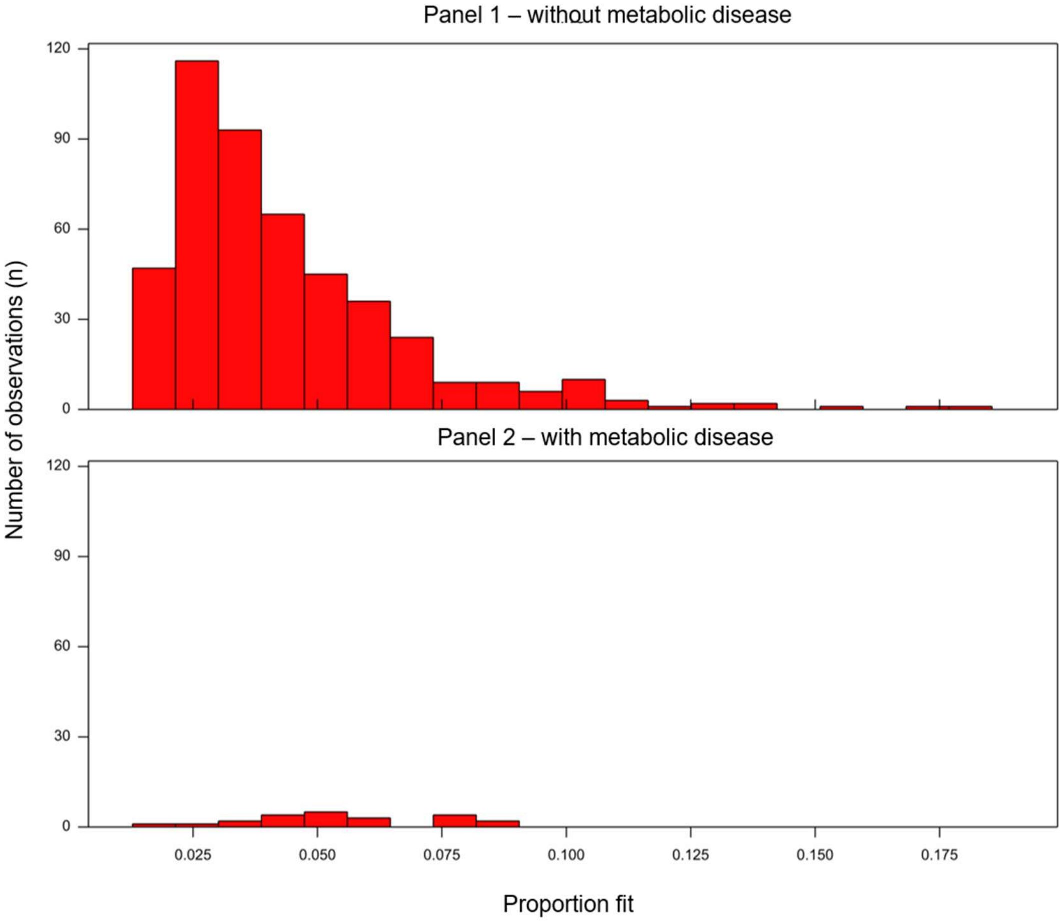


Figure 4.18: Histograms of proportion of cow-lactations with metabolic disorders estimated from GLMM with body weight at dry off included as a fixed effect for cow-lactations without (Panel 1) and without (Panel 2) metabolic disorders

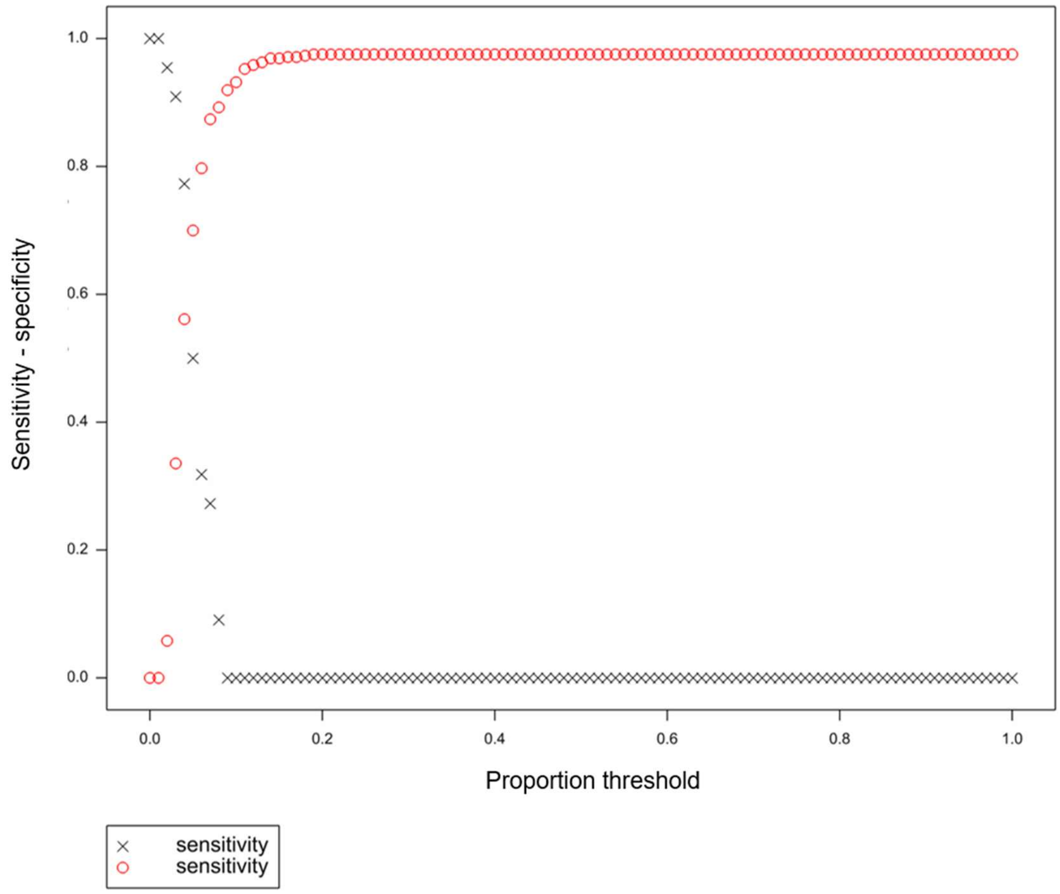


Figure 4.19: Sensitivity and specificity based on thresholds of proportion of cow-lactations with metabolic disorders in early lactation estimated from GLMM with body weight at dry off included as a fixed effect

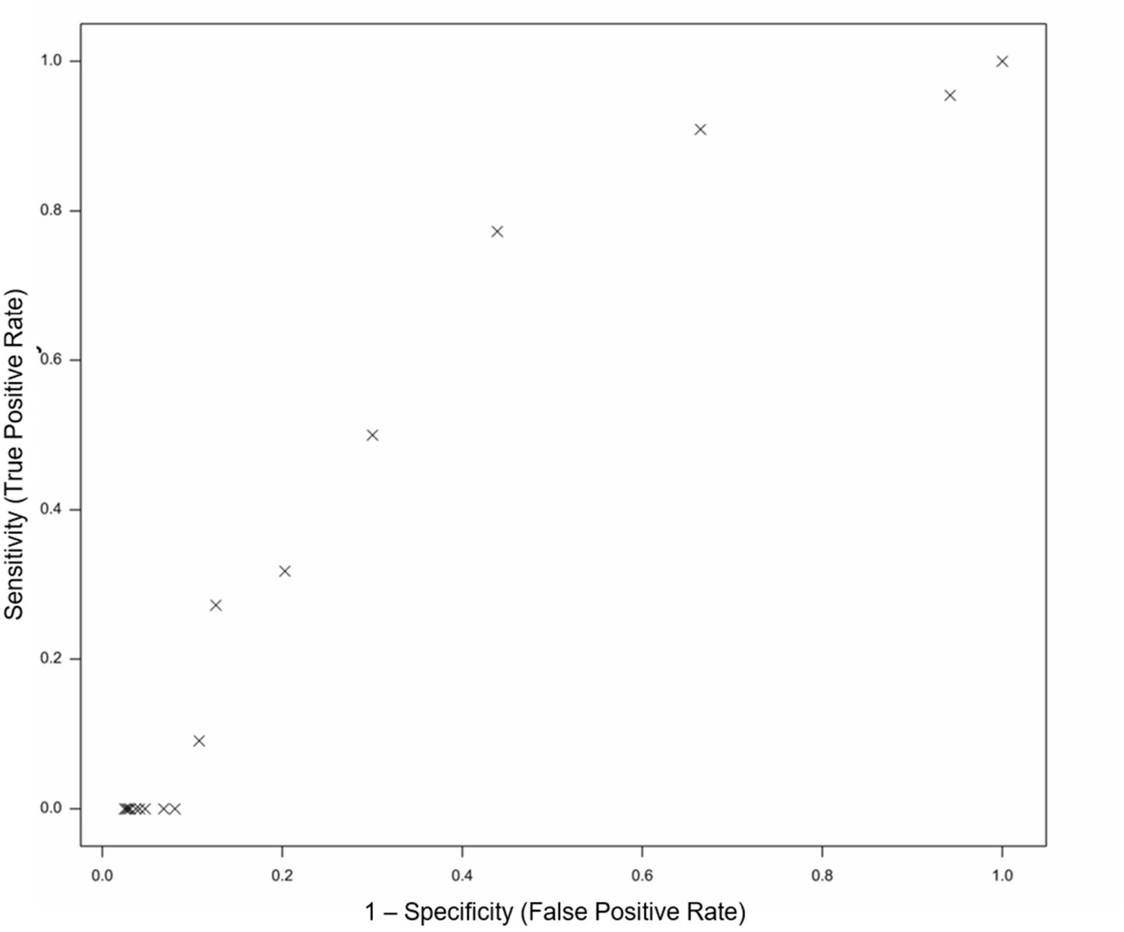


Figure 4.20: ROC Curve based on thresholds of proportions of cow-lactations with metabolic disorders in early lactation estimated from GLMM with body weight at dry off included as a fixed effect (Optimum sensitivity = 0.30, optimum specificity = 0.69)

Like the results seen for reproductive disorders, none of the candidate indicators for metabolic disease were found to be good classifiers of metabolic disease despite mean values for these traits being significantly different between cows with metabolic disease and cows without metabolic disease.

(iii) Subclinical mastitis – GLMM & assessing model performance

All GLMM models for subclinical mastitis ran successfully but no candidate variables were found to be significant at $p< 0.05$ (Table 4.6). Of note is the fact that milk yield at dry off was not found to be significant as a predictor, due to very large standard errors associated with each mean predicted probability (Figure 4.21).

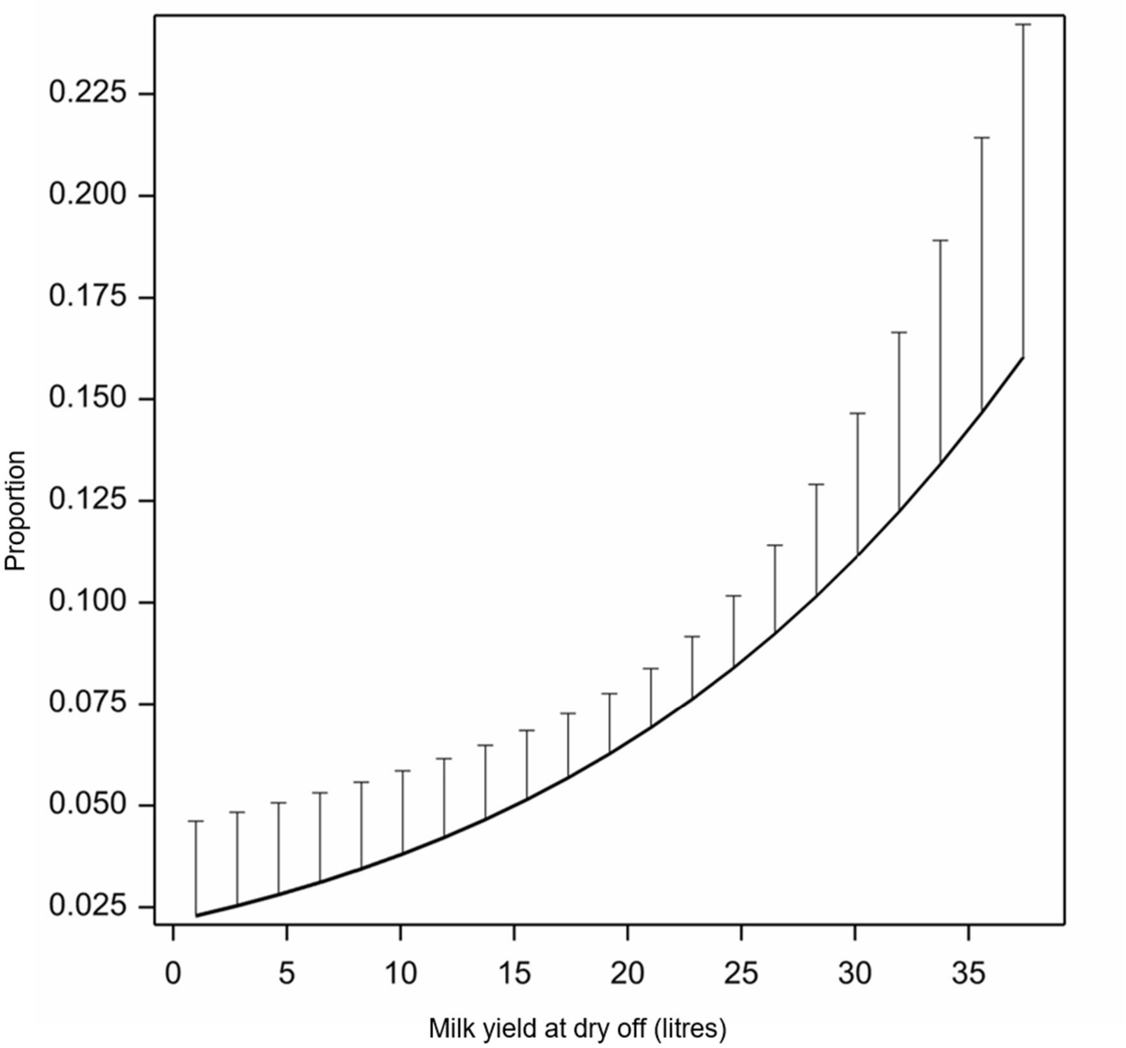


Figure 4.21: Proportion of cow-lactations with mastitis estimated from GLMM with milk yield at dry off included as a fixed effect shown as a function of milk yield at dry off

Table 4.6: Individual cow variance components with associated standard error and test statistics from GLMMs for subclinical mastitis with each variable included as a fixed effect and cow as a random effect

Variable type	**Variable**	**Individual cow variance component**	**Standard error of variance component**	**Wald Statistic**	**Numerator degrees of freedom**	**p-value (Wald)**	**F-statistic**	**Denominator degrees of freedom**	**p-value (F-statistic)**	**P-value**	**Direction of effect for p<0.05**
Factor	Cycle number	0.00000		0.006	1.0	0.940	0.006	501.0	0.941	0.941	
Factor	Month of dry off	0.00000		6.795	11.0	0.815	0.618	491.0	0.814	0.814	
Factor	Month of calving	0.00000		9.100	11.0	0.613	0.827	491.0	0.613	0.613	
Factor	Genetic line	0.00000		0.737	1.0	0.391	0.737	501.0	0.391	0.391	
Factor	Diet type	0.00000		1.218	1.0	0.270	1.218	501.0	0.270	0.270	
Covariate	Year of dry off	0.00000		3.760	1.0	0.053	3.760	501.0	0.053	0.053	
Covariate	Year of calving	0.00000		3.633	1.0	0.057	3.633	501.0	0.057	0.057	
Covariate	Dry period length	0.00000		2.243	1.0	0.134	2.243	501.0	0.135	0.135	
Covariate	BW at dry off	0.00000		0.145	1.0	0.703	0.145	489.0	0.703	0.703	
Covariate	BW at calving	0.00000		1.665	1.0	0.197	1.665	488.0	0.198	0.198	
Covariate	BW change	0.00000		1.538	1.0	0.215	1.538	476.0	0.216	0.216	
Covariate	BW intercept (calving)	0.00000		2.963	1.0	0.085	2.963	472.0	0.086	0.086	
Covariate	BW intercept (-60)	0.00000		0.665	1.0	0.415	0.665	472.0	0.415	0.415	
Covariate	CS at dry off	0.00000		0.062	1.0	0.803	0.062	501.0	0.803	0.803	
Covariate	CS change	0.00000		0.387	1.0	0.534	0.387	496.0	0.534	0.534	
Covariate	CS intercept (-60)	0.00000		1.037	1.0	0.308	1.037	490.0	0.309	0.309	
Covariate	CS slope	0.00000		1.290	1.0	0.256	1.290	490.0	0.257	0.257	
Covariate	BEC at dry off	0.00000		0.269	1.0	0.604	0.269	489.0	0.604	0.604	
Covariate	BEC at calving	0.00000		0.176	1.0	0.675	0.176	483.0	0.675	0.675	
Covariate	BEC change	0.00000		0.023	1.0	0.878	0.023	471.0	0.878	0.878	
Covariate	BEC intercept (calving)	0.00000		2.190	1.0	0.139	2.190	445.0	0.140	0.140	
Covariate	BEC intercept (-60)	0.00000		0.637	1.0	0.425	0.637	445.0	0.425	0.425	
Covariate	BEC slope	0.00000		1.366	1.0	0.242	1.366	445.0	0.243	0.243	
Covariate	Yield at dry off	0.00027	0.90524	3.212	1.0	0.073	3.212	237.1	0.074	0.074	

* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001

(iv) All disease – GLMM & assessing model performance

In addition to running GLMM models for each health outcome, all health groups (REP, MET, MAST) were combined and compared with cows that remained healthy (NCD) after calving; all models ran successfully (Table 4.6). Genetic line was the only factor that was significant (p= 0.021) with Select cows at greater risk of disease in early lactation compared to Control cows (23% versus 33%, respectively) as seen in Figure 4.22.

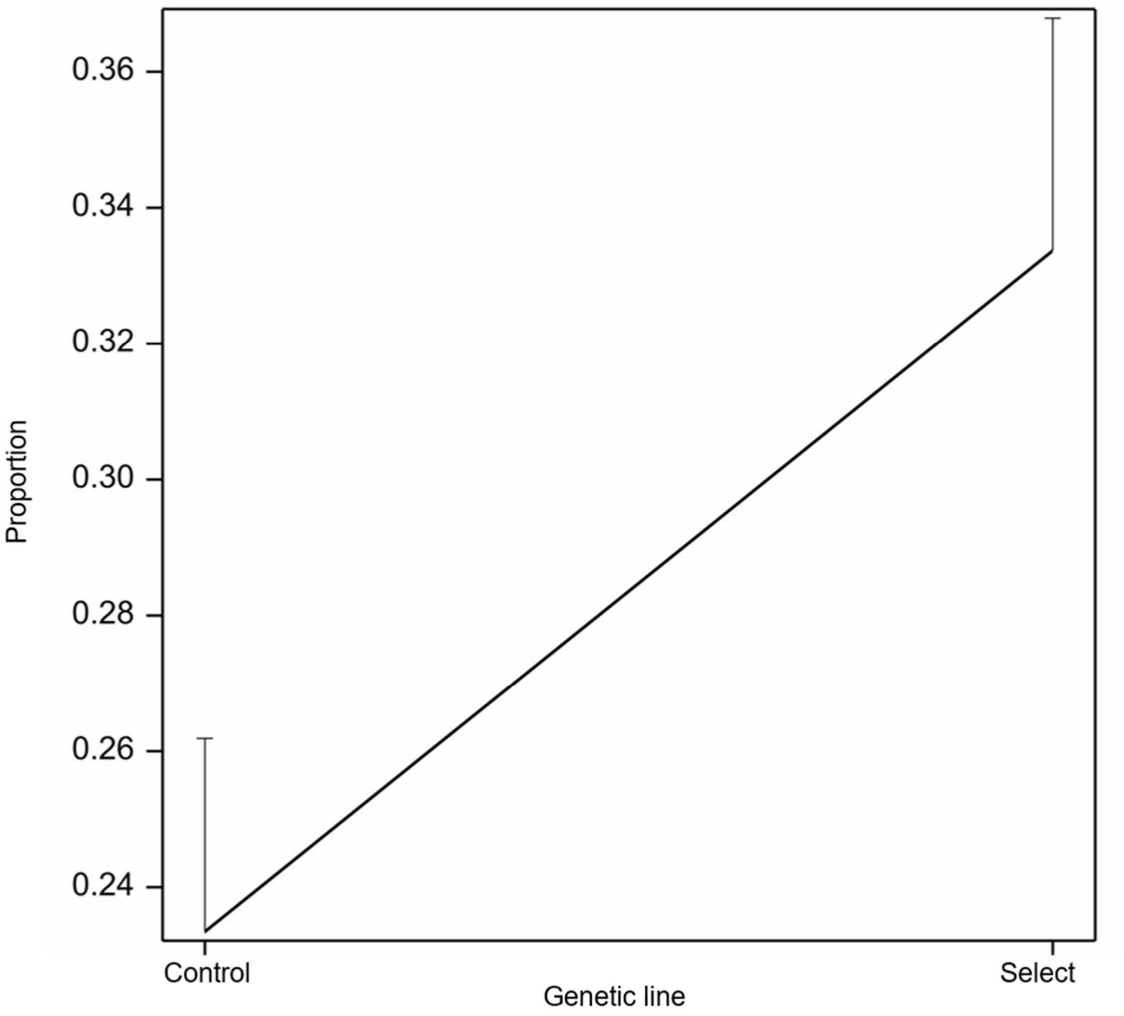


Figure 4.22: Proportion of cow-lactations with disease estimated from GLMM with genetic line included as a fixed effect shown as a function of genetic line

In addition to genetic line, 12 candidate indicators were found to be significant; dry period length (p= 0.005), BW at dry off (p= 0.001), BW at calving (p= 0.04), BW change (p= 0.013), BW intercept at calving (p= 0.000), BW intercept 60 days before calving (p= 0.002), CS at dry off (p= 0.044), CS change (p= 0.001), BEC at dry off (p= 0.003), BEC change (p=0.001), BEC intercept at calving (p= 0.001) and BEC intercept 60 days before calving (p= 0.002).

Condition score at dry off was found to be significant for the risk of any disease (p=0.044). Figure 4.23 shows that the proportion of cows with any disease increases from 27.5% for cows with a body condition score of 2.5 at drying to 40% for cows with a body condition score of 3.5 at drying. As seen in the other examples provided, despite the large and statistically significant effects seen (Figure 4.23), the fit of the model was poor due to the large overlap in the distributions between cow- lactations with disease and those without disease (Figure 4.24). A flatter distribution is seen in the cows with disease which means there are a higher proportion of cows within this population at the higher end of the range, however the sample size is small. Thus, the specificity and sensitivity of condition score at dry off as a predictor for all disease in early lactation was poor (Figure 4.25 & Figure 4.26).

Table 4.6: Individual cow variance components with associated standard error and test statistics from GLMMs for combined health group (binany) with each variable included as a fixed effect and cow as a random effect

Variable type	Variable	Individual cow variance component	Standard error of variance component	Wald Statistic	Numerator degrees of freedom	p-value (Wald)	F-statistic	Denominator degrees of freedom	p-value (F-statistic)	P-value	Direction of effect for p<0.05
Factor	Cycle number	0.47458	0.28039	3.313	1.0	0.069				0.069	
Factor	Month of dry off	0.48361	0.28780	4.986	11.0	0.932				0.932	
Factor	Month of calving	0.54795	0.29412	9.983	11.0	0.532				0.532	
Factor	Genetic line	0.43172	0.27875	5.402	1.0	0.020	5.402	326.7	0.021	0.021	S>C
Factor	Diet type	0.46291	0.27843	0.650	1.0	0.420	0.650	331.1	0.421	0.421	
Covariate	Year of dry off	0.45965	0.27778	0.914	1.0	0.339	0.914	455.9	0.340	0.340	
Covariate	Year of calving	0.45790	0.27787	1.296	1.0	0.255	1.296	448.2	0.256	0.256	
Covariate	Dry period length	0.47548	0.28455	8.017	1.0	0.005				0.005	Positive
Covariate	BW at dry off	0.48259	0.29171	11.766	1.0	0.001				0.001	Positive
Covariate	BW at calving	0.41137	0.28120	4.202	1.0	0.040				0.040	Positive
Covariate	BW change	0.45173	0.29442	6.152	1.0	0.013				0.013	Negative
Covariate	BW intercept (calving)	0.37242	0.28772	12.265	1.0	0.000				0.000	Positive
Covariate	BW intercept (-60)	0.40942	0.28928	9.970	1.0	0.002				0.002	Positive
Covariate	CS at dry off	0.45808	0.27914	4.067	1.0	0.044				0.044	Positive
Covariate	CS change	0.40328	0.28007	10.244	1.0	0.001				0.001	Negative
Covariate	CS intercept (-60)	0.50819	0.28825	1.784	1.0	0.182				0.182	
Covariate	CS slope	0.49773	0.28751	1.641	1.0	0.200				0.200	
Covariate	BEC at dry off	0.48501	0.29035	8.536	1.0	0.003				0.003	Positive
Covariate	BEC at calving	0.39988	0.28120	1.274	1.0	0.259				0.259	
Covariate	BEC change	0.40211	0.29536	11.921	1.0	0.001				0.001	Negative
Covariate	BEC intercept (calving)	0.41494	0.30427	11.274	1.0	0.001				0.001	Positive
Covariate	BEC intercept (-60)	0.42906	0.30418	10.027	1.0	0.002				0.002	Positive
Covariate	BEC slope	0.43809	0.30025	0.014	1.0	0.907				0.907	
Covariate	Yield at dry off	0.36878	0.32083	0.510	1.0	0.475	0.510	358.8	0.476	0.476	

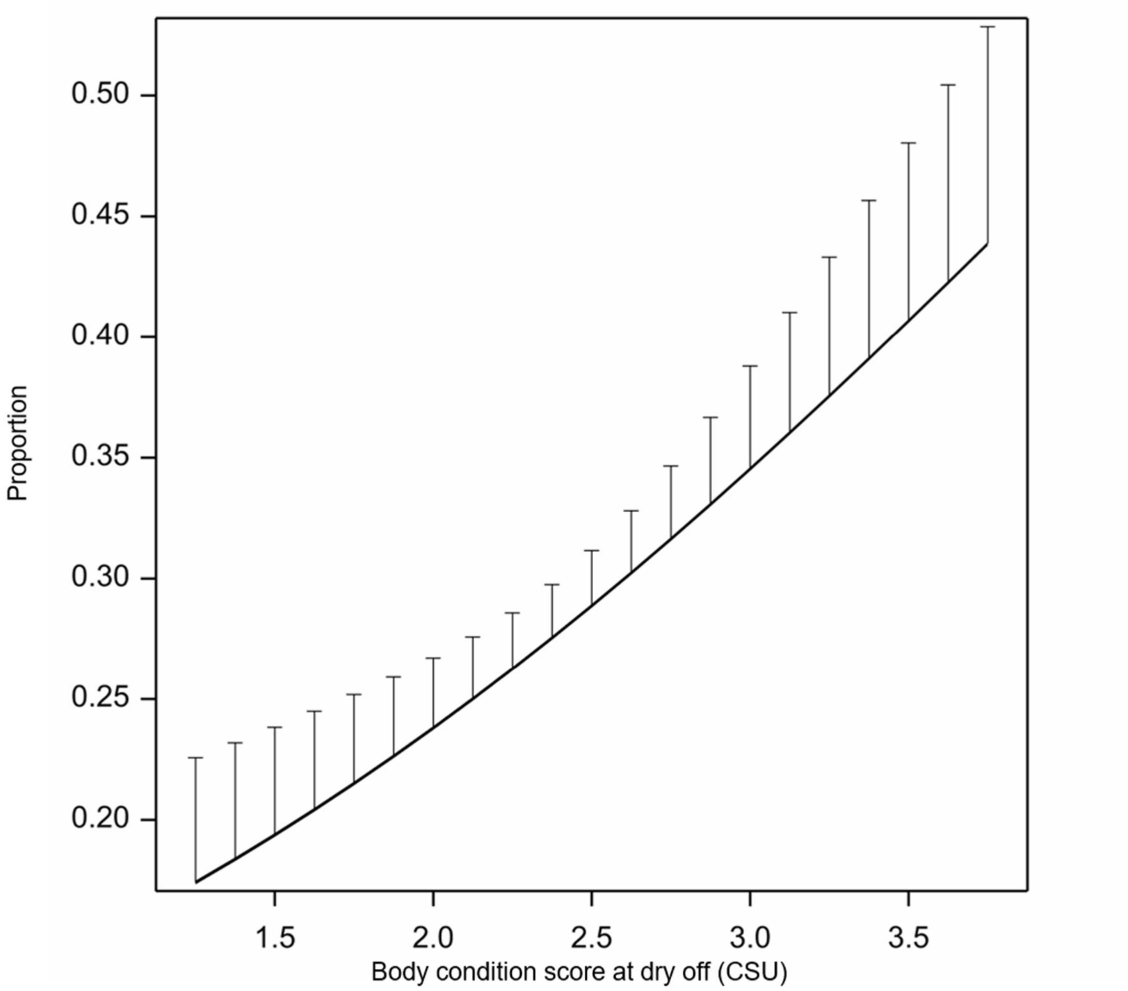


Figure 4.23: Proportion of cow-lactations with any disease estimated from GLMM with body condition score at calving included as a fixed effect shown as a function of body condition score at calving

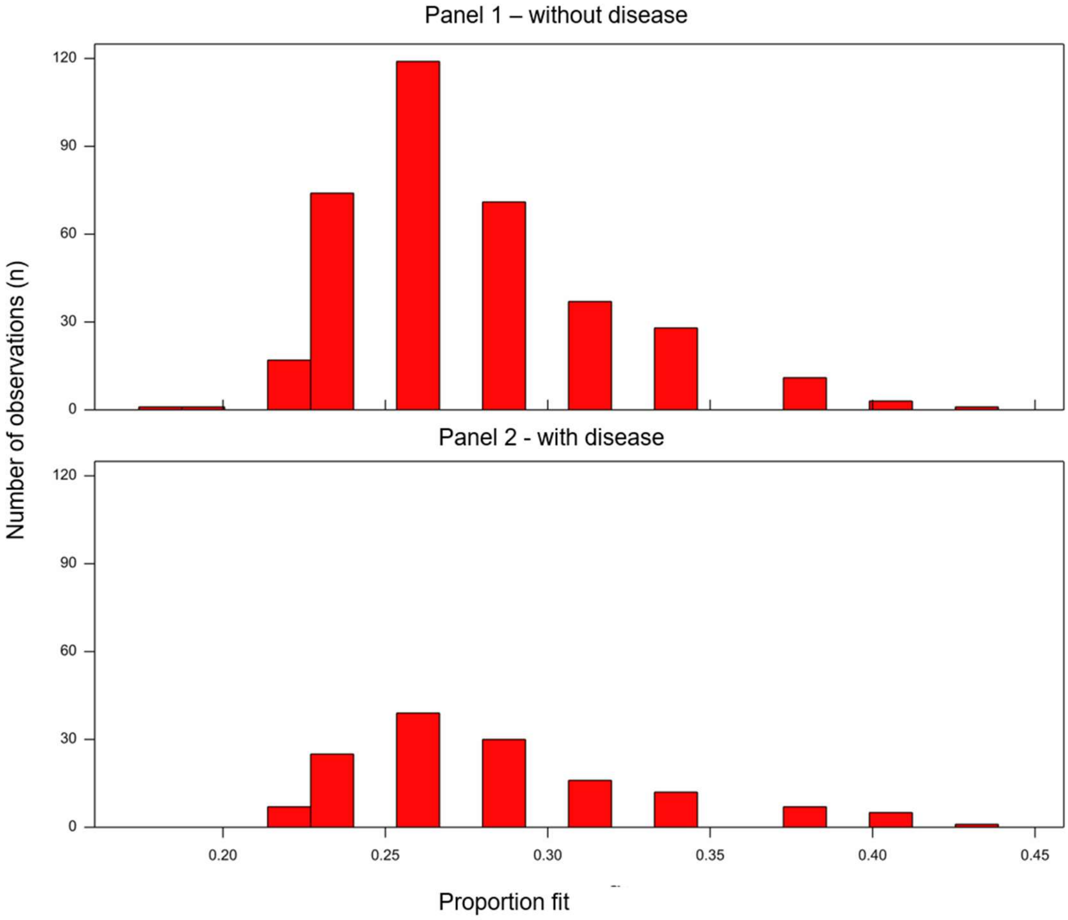


Figure 4.24: Histograms of proportion of cow-lactations with any disease estimated from GLMM with body condition score at drying included as a fixed effect for cow-lactations with (Panel 1) and without (Panel 2) any disease

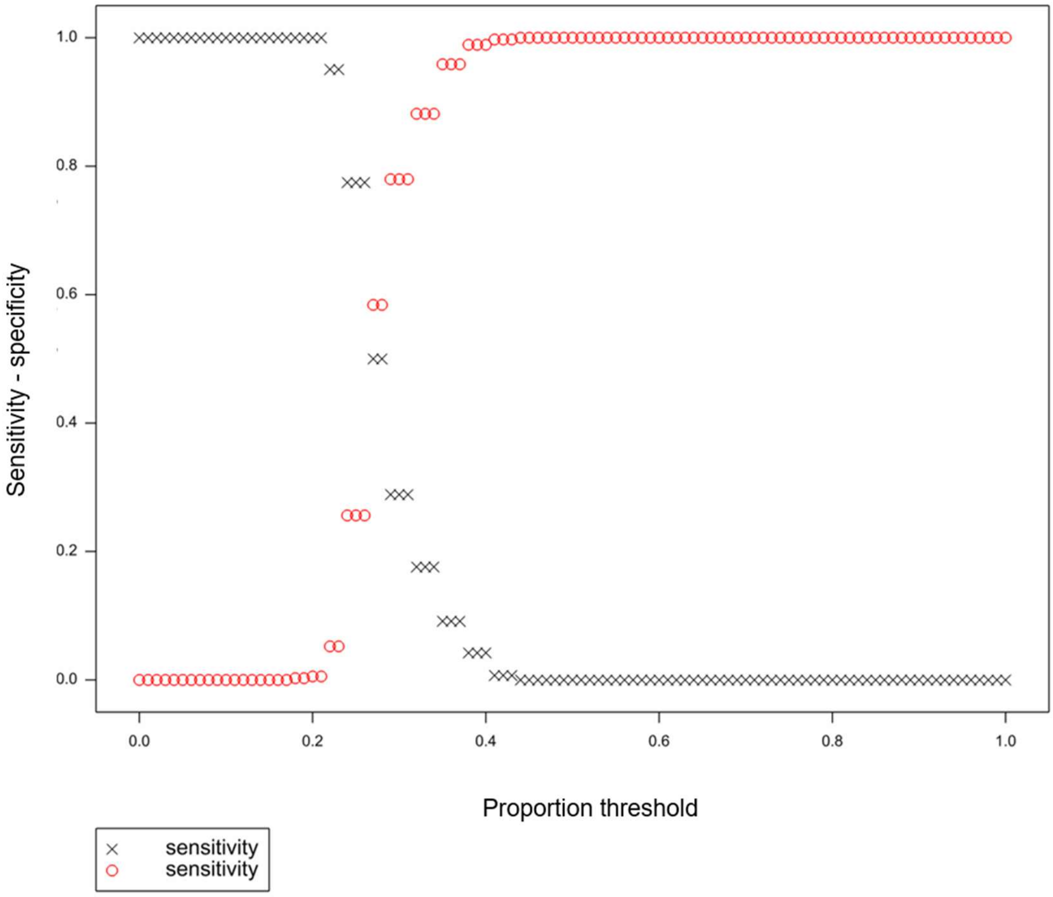


Figure 4.25: Sensitivity and specificity based on thresholds of proportion of cow-lactations with any disease in early lactation estimated from GLMM with body condition score at calving included as a fixed effect

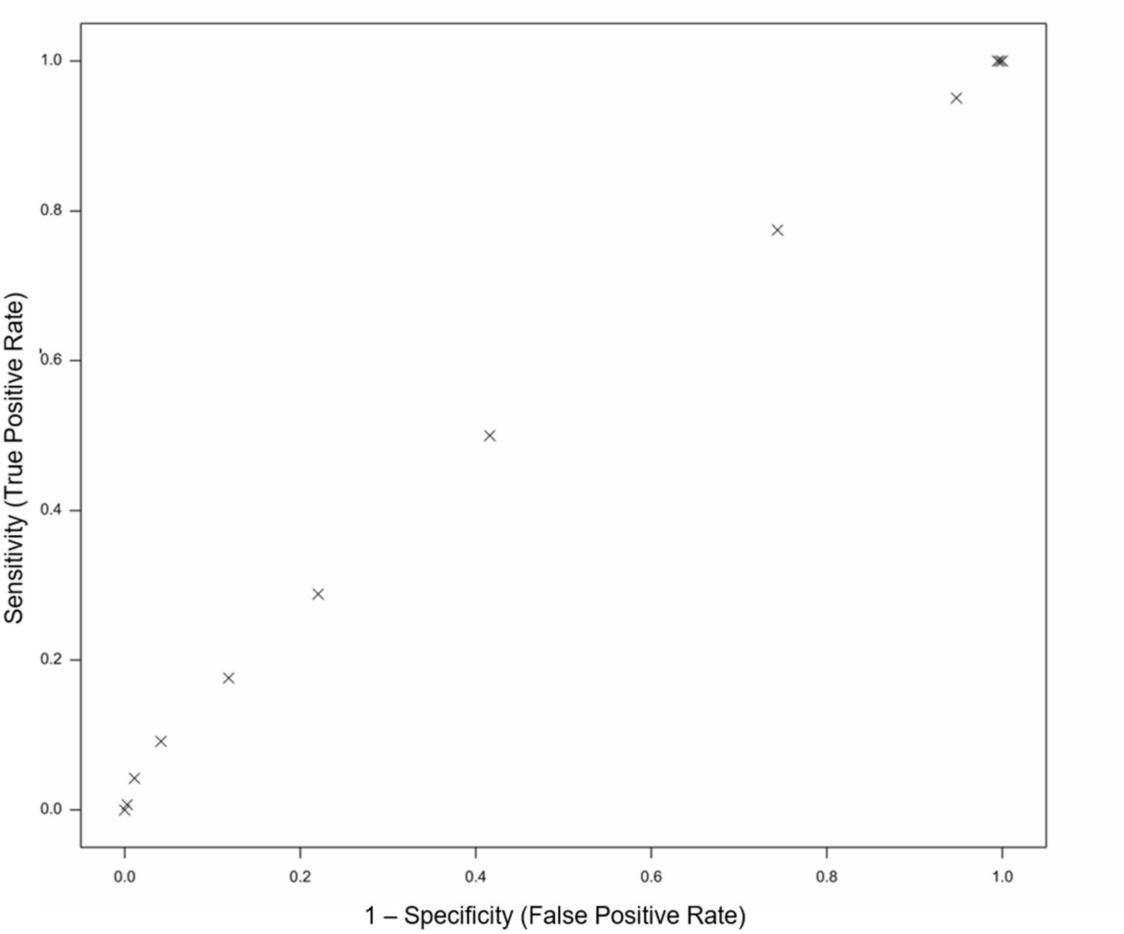


Figure 4.26: ROC Curve based on thresholds of proportions of cow-lactations with any disease in early lactation estimated from GLMM with body condition score at drying included as a fixed effect (Optimum sensitivity = 0.50, optimum specificity = 0.58)

A further example of a candidate indicator which was found to be significant for the risk of any disease is body weight change (p= 0.013), the effect of which is negative on mean predicted probability. Figure 4.27 shows that the proportion of cows with any disease decreases from over 60% for cows with a body weight change of -6kg per day, over the dry period, to 10% for cows with a body weight change of +4kg per day. Cows that did not change in body weight (i.e. those cows which had body weight change of 0kg/day) had a mean predicted probability of disease of approximately 25%. Despite this significant effect Figure 4.28 illustrates that the model fit was poor due to the large coincidence in the range of observed values of body weight change in the two populations (cow-lactations with disease and those without disease). Visually there appears to be a higher proportion of cows within this population of cows with disease at the higher end of the range, however the sample size is very small. Thus, the specificity and sensitivity of body weight change as a predictor for all disease in early lactation was also found to be poor (Figure 4.29 & Figure 4.30). The ROC curve is close to diagonal and therefore the model is not good at identifying either cows that have disease or cows that do not have disease.

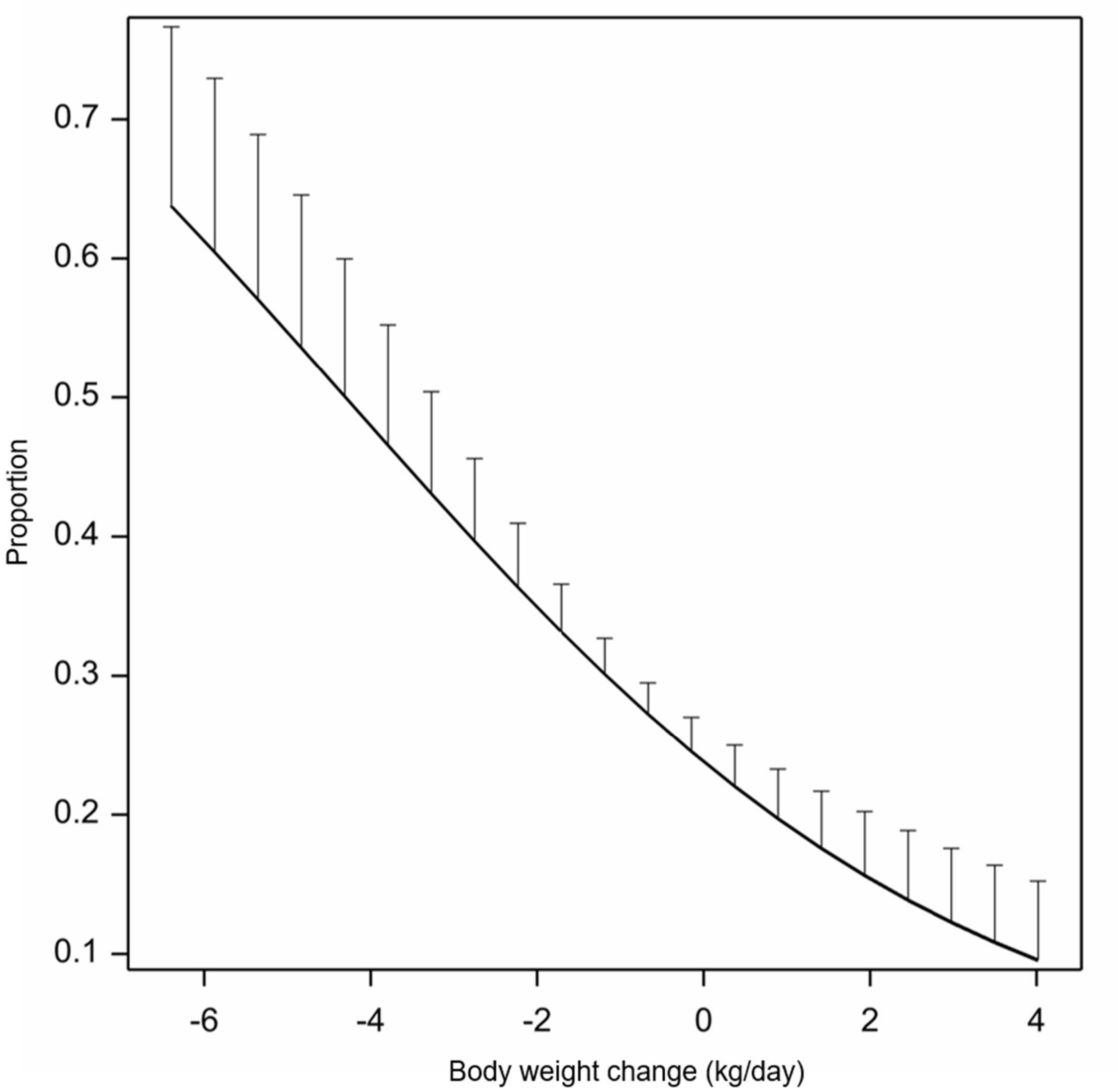


Figure 4.27: Proportion of cow-lactations with any disease estimated from GLMM with body weight change included as a fixed effect shown as a function of body weight change

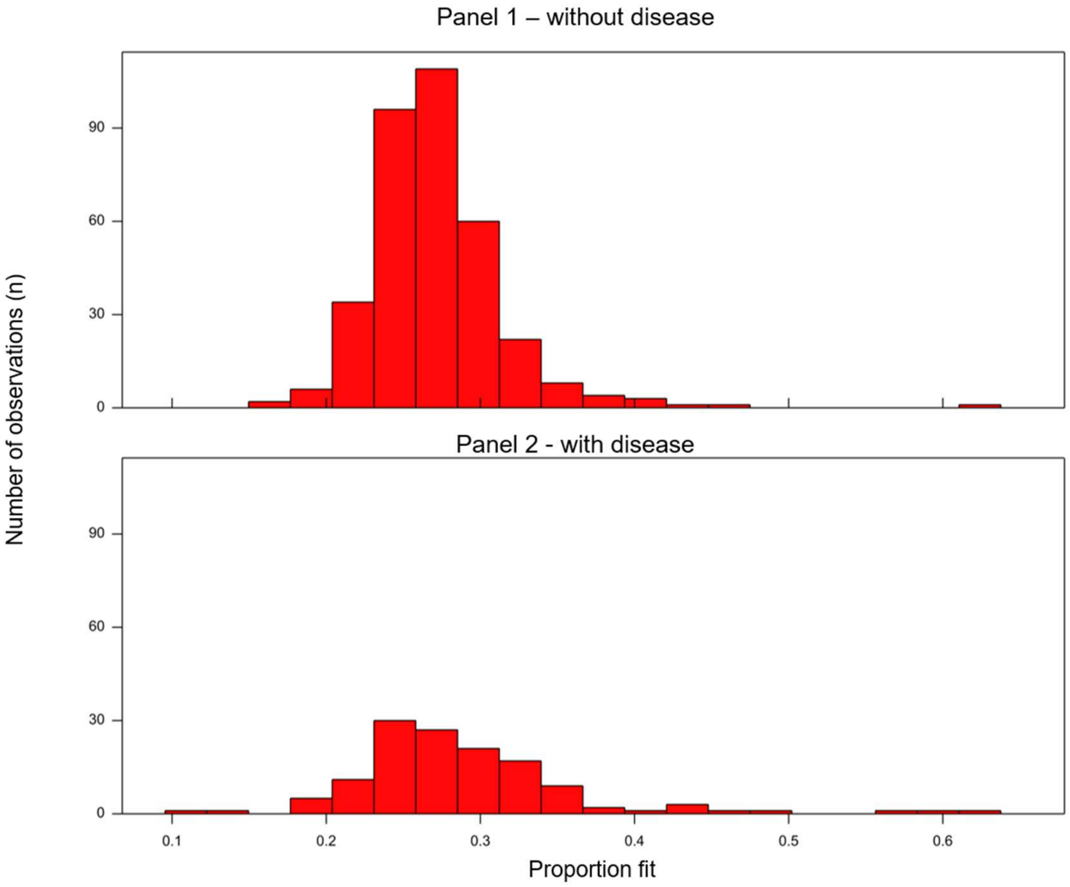


Figure 4.28: Histograms of proportion of cow-lactations with any disease estimated from GLMM with body weight change included as a fixed effect for cow-lactations with (Panel 1) and without (Panel 2) any disease

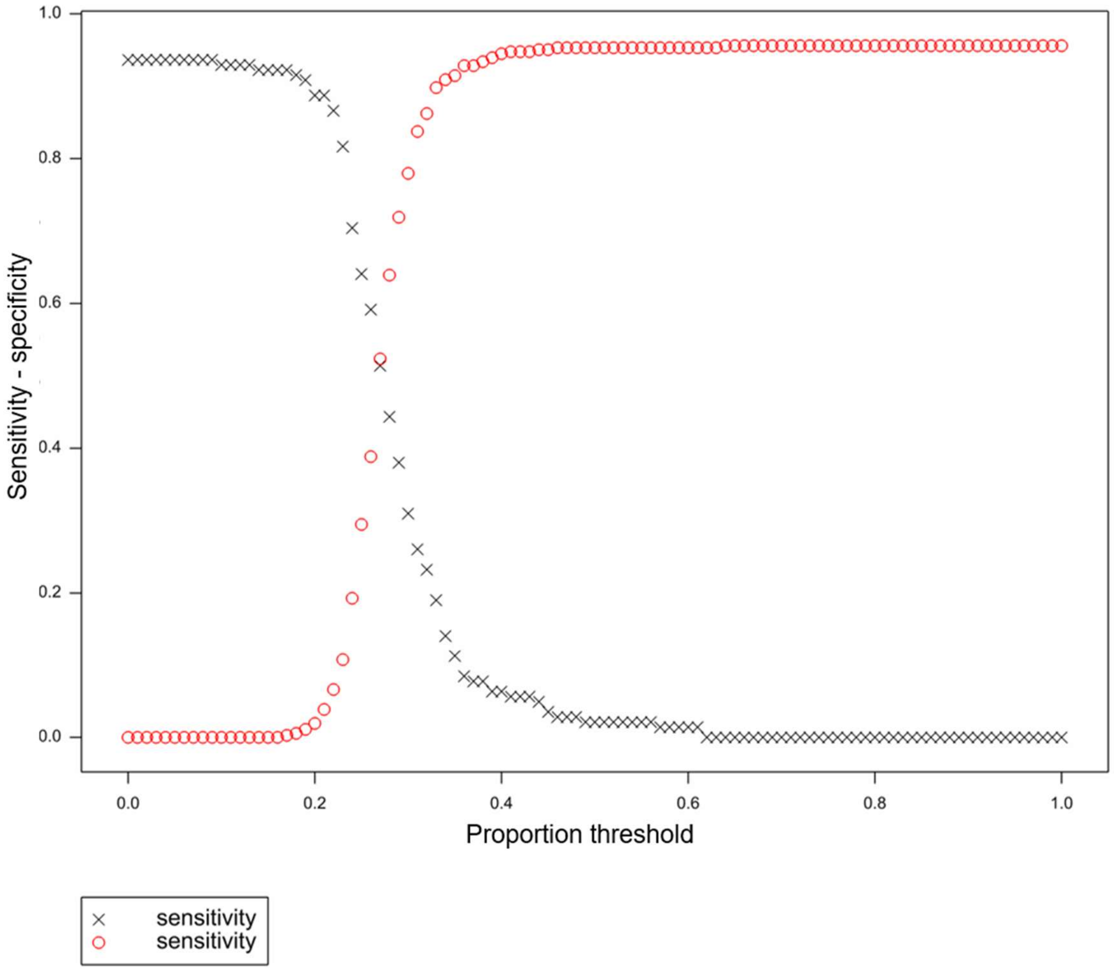


Figure 4.29: Sensitivity and specificity based on thresholds of proportion of cow-lactations with any disease in early lactation estimated from GLMM with body weight change included as a fixed effect

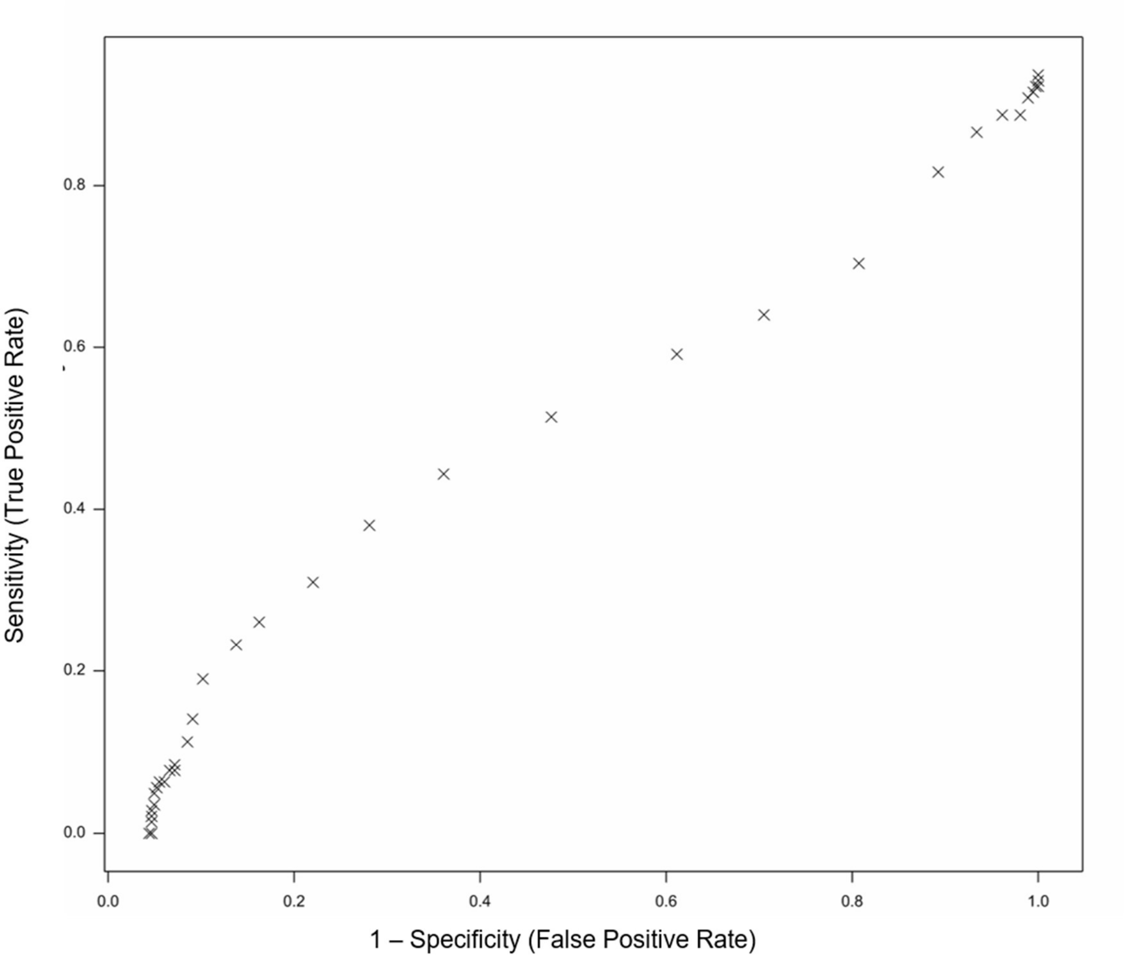


Figure 4.30: ROC Curve based on thresholds of proportions of cow-lactations with any disease in early lactation estimated from GLMM with body weight change included as a fixed effect (Optimum sensitivity = 0.38, optimum specificity = 0.71)

4.4.4 Multivariate models

Multivariate models including all variables that were significant at p<0.1 were run for each health outcome.

(i) Reproductive disorders

The multivariate model generated for reproductive disorders included 13 candidate indicator variables which had been found to be significant (p<0.1) when included in single variable models in the previous stage of the analysis.

Table 4.7: Test statistics for multivariate model including all variables significant at p<0.1 in single variable models for reproductive disorders.

Variable	**Wald-statistic**	**Numerator degrees of freedom**	**p-value (Wald)**
BEC change	11.47163	1	0.001
BW change	0.52268	1	0.470
CS change	0.14233	1	0.706
BW at dry off	0.08134	1	0.775
CS at dry off	0.00808	1	0.928
CS intercept (-60)	0.00068	1	0.979
BEC at drying	0.00110	1	0.974
Dry period length	5.92157	1	0.015
BEC intercept (-60)	3.04360	1	0.081
CS slope	1.17118	1	0.279
BW intercept (-60)	0.00515	1	0.943
BEC intercept (calve)	2.35764	1	0.125
BW intercept (calve)	0.00221	1	0.963

BEC change is significant at a similar level as to when it was fitted on its own, thereafter the only variable which remains significant when entered the model sequentially is dry period length p = 0.015. Figure 4.31 shows that there is no discrimination between the populations of cows with and without reproductive disorders. Consequently, the specificity and sensitivity of the model is poor (Figure 4.32 & Figure 4.33).

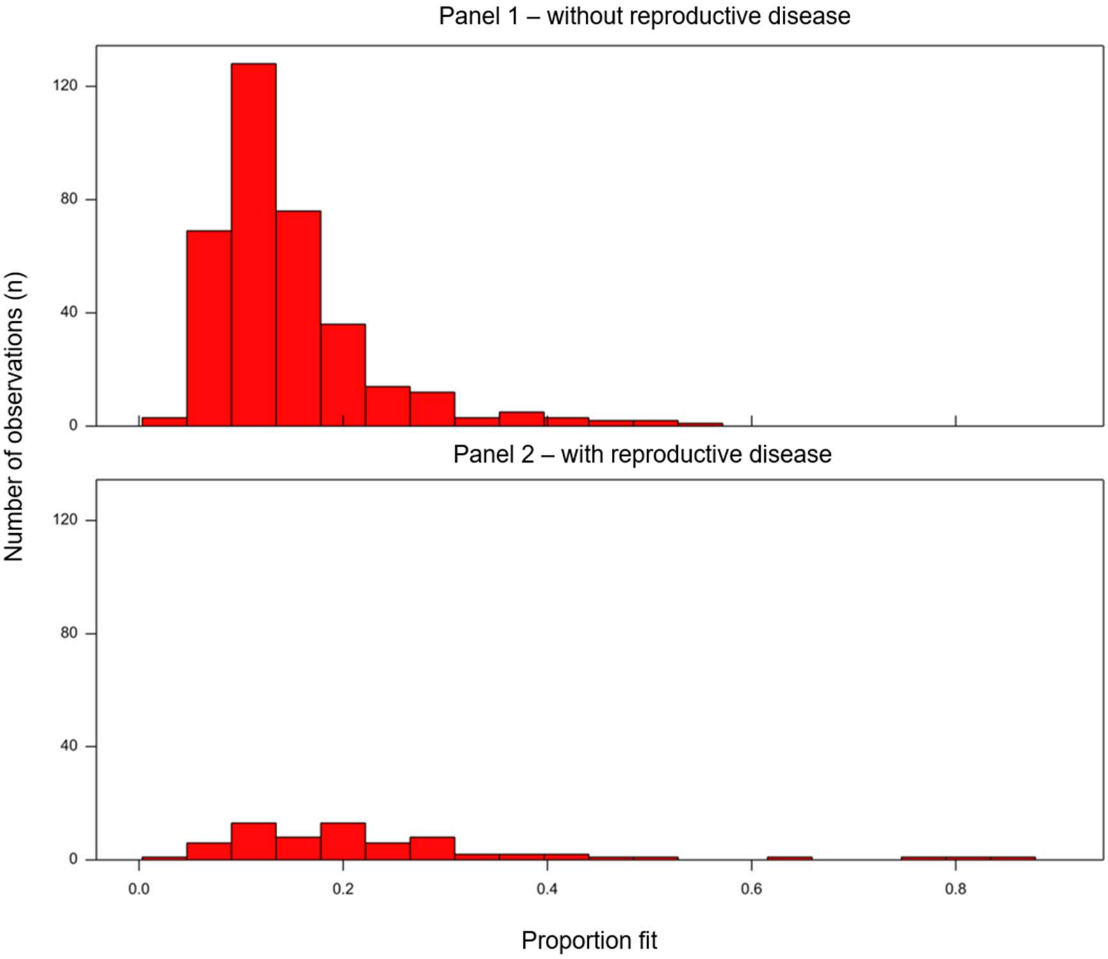


Figure 4.31: Histograms of proportion of cow-lactations with reproductive disorders estimated from multivariate GLMM for cow-lactations with (Yes) and without (No) reproductive disorders

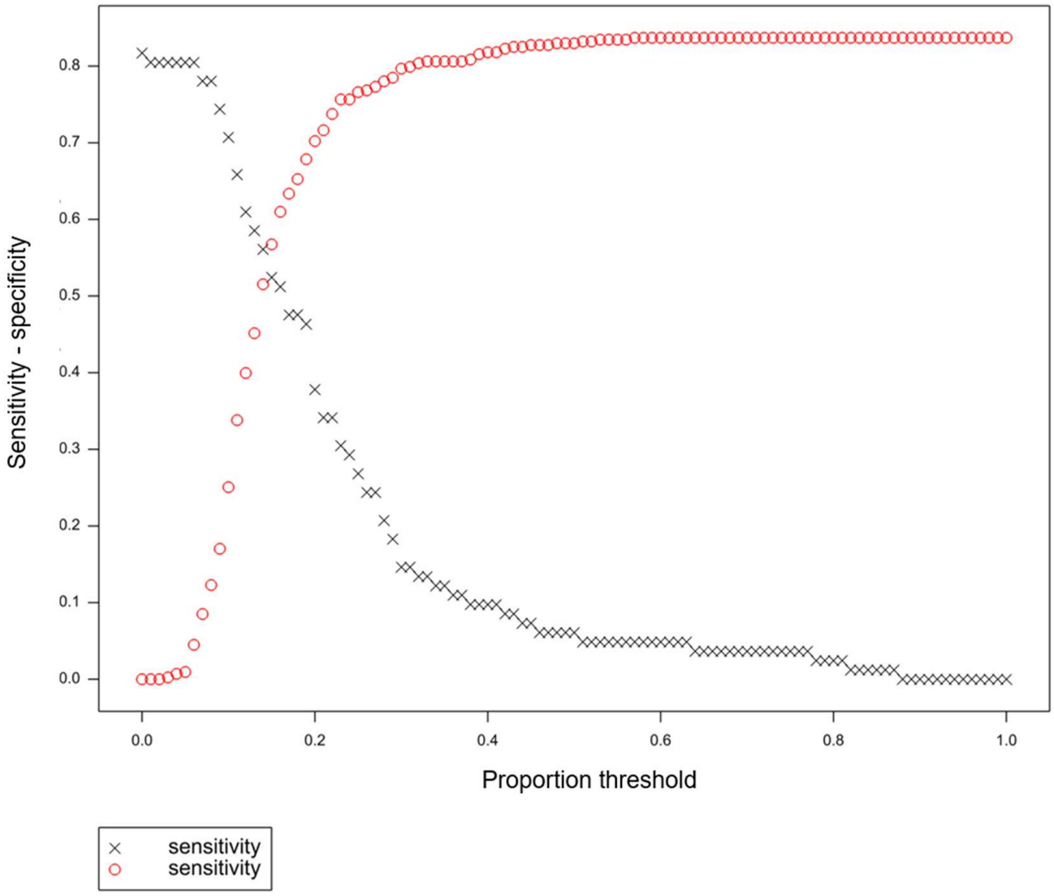


Figure 4.32: Sensitivity and specificity based on thresholds of proportion of cow-lactations with reproductive disorders in early lactation estimated from GLMM with body condition score at calving included as a fixed effect

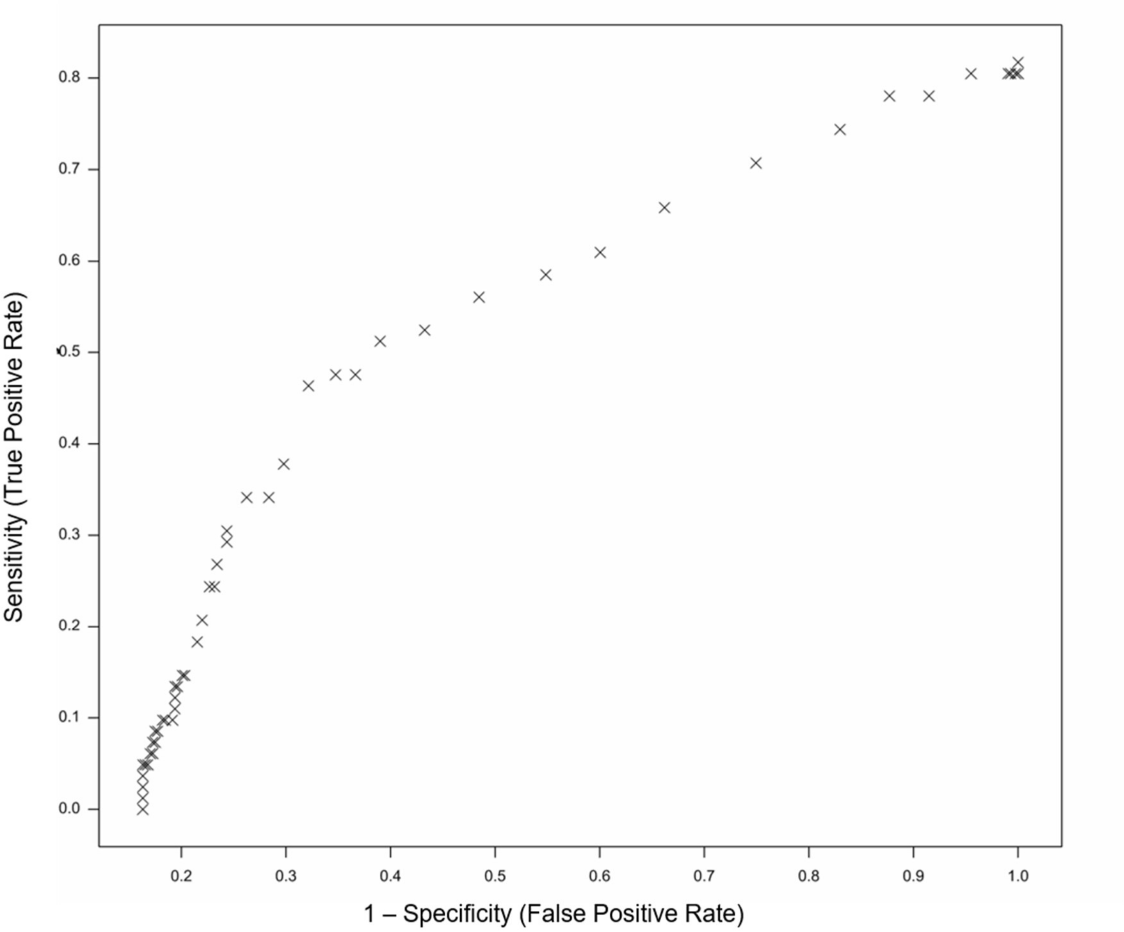


Figure 4.33: ROC Curve based on thresholds of proportions of cow-lactations with any disease in early lactation estimated from GLMM with body condition score at drying included as a fixed effect (Optimum sensitivity = 0.52, optimum specificity = 0.56)

(ii) Metabolic disorders

The multivariate model generated for metabolic disorders included 5 candidate indicator variables which had been found to be significant ($p<0.1$) when included in single variable models in the previous stage of the analysis (Table 4.8).

Table 4.8: Test statistics for multivariate model including all variables significant at $p<0.1$ in single variable models for metabolic disorders

Variable	**Wald-statistic**	**Numerator degrees of freedom**	**p-value (Wald)**
Parity	5.24391	1	0.022
BW intercept (calving)	1.73340	1	0.187
BW at drying	0.97777	1	0.322
BW intercept (-60)	1.96744	1	0.160
BEC intercept (calving)	0.67127	1	0.412

Parity is significant ($p=0.02$) when included as an explanatory variable for metabolic disorders but all other variables are insignificant and are therefore not enhancing the model's explanatory ability. Figure 4.34 shows that there is no discrimination between the populations of cows with and without metabolic disorders. Consequently, the specificity and sensitivity of the model is poor (Figure 4.35 & Figure 4.36).

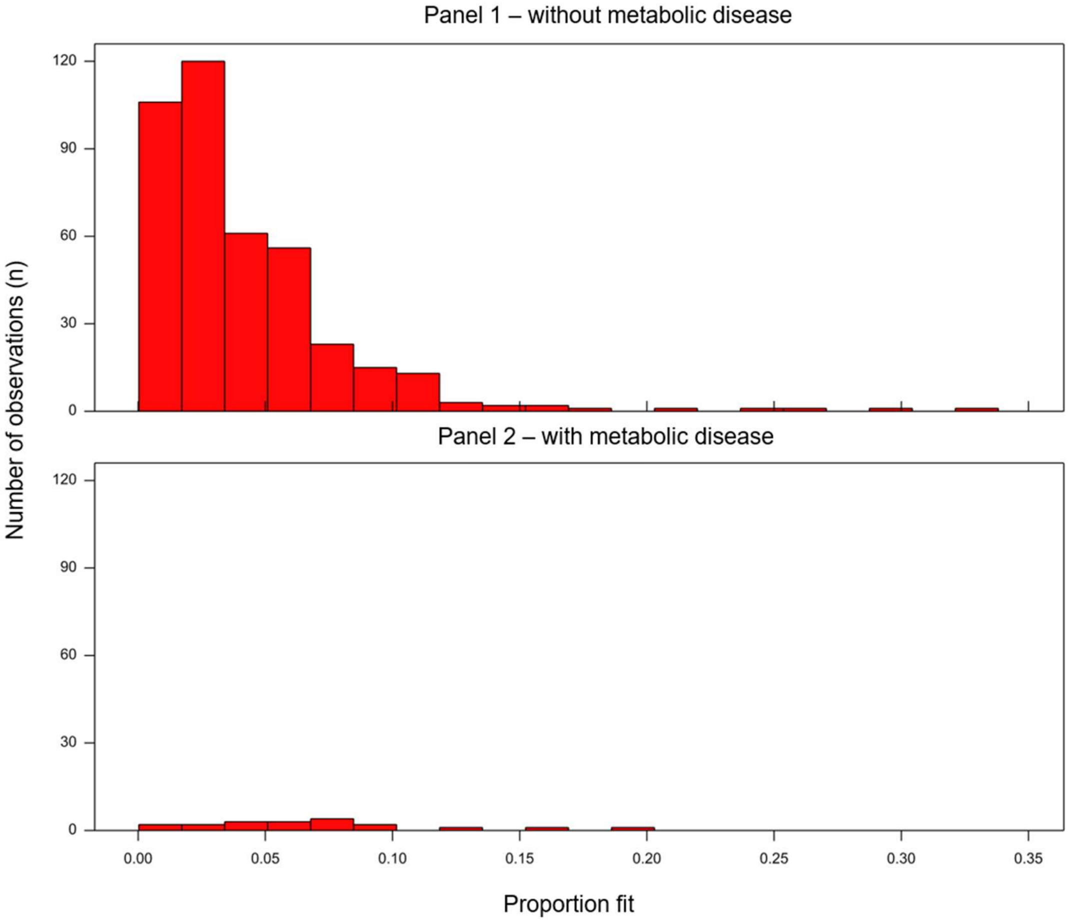


Figure 4.34: Histograms of proportion of cow-lactations with metabolic disorders estimated from multivariate GLMM for cow-lactations with (Yes) and without (No) reproductive disorders

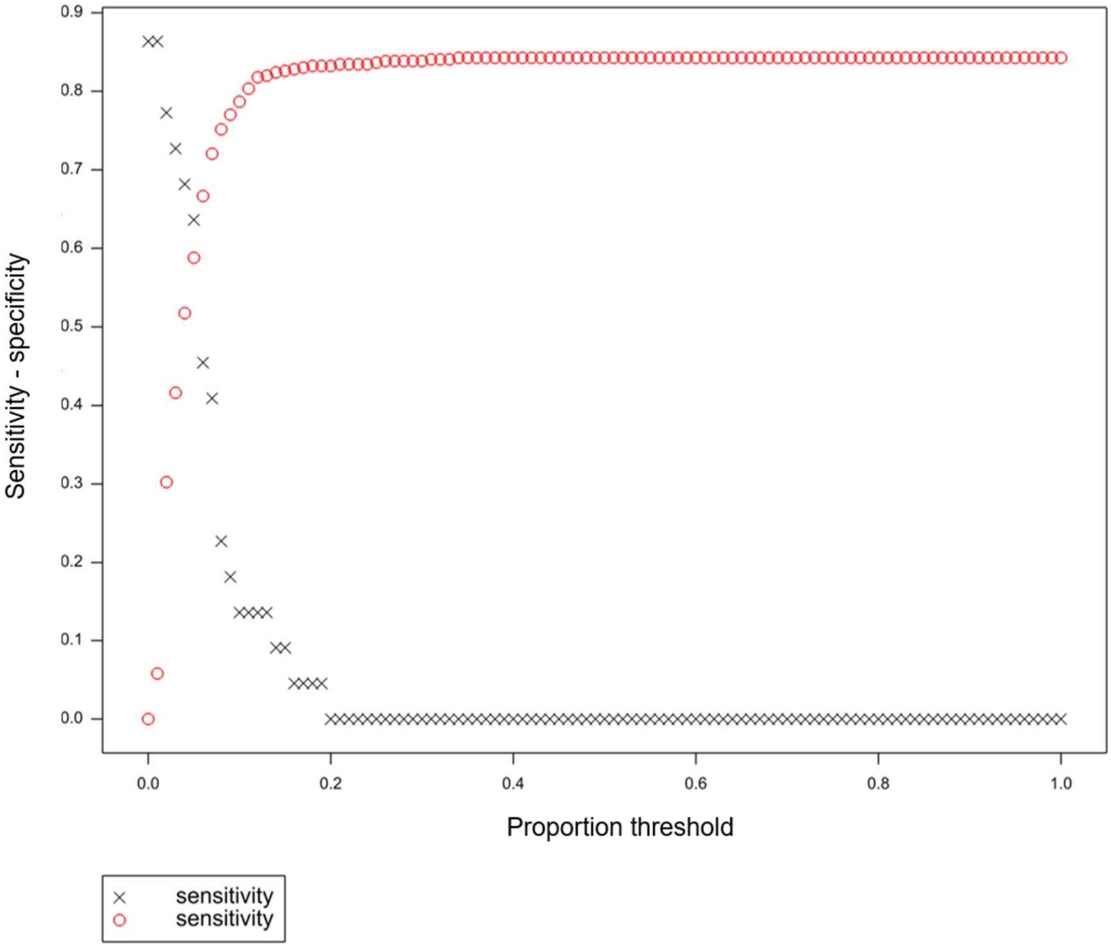


Figure 4.35: Sensitivity and specificity based on thresholds of proportion of cow-lactations with metabolic disorders in early lactation estimated from GLMM

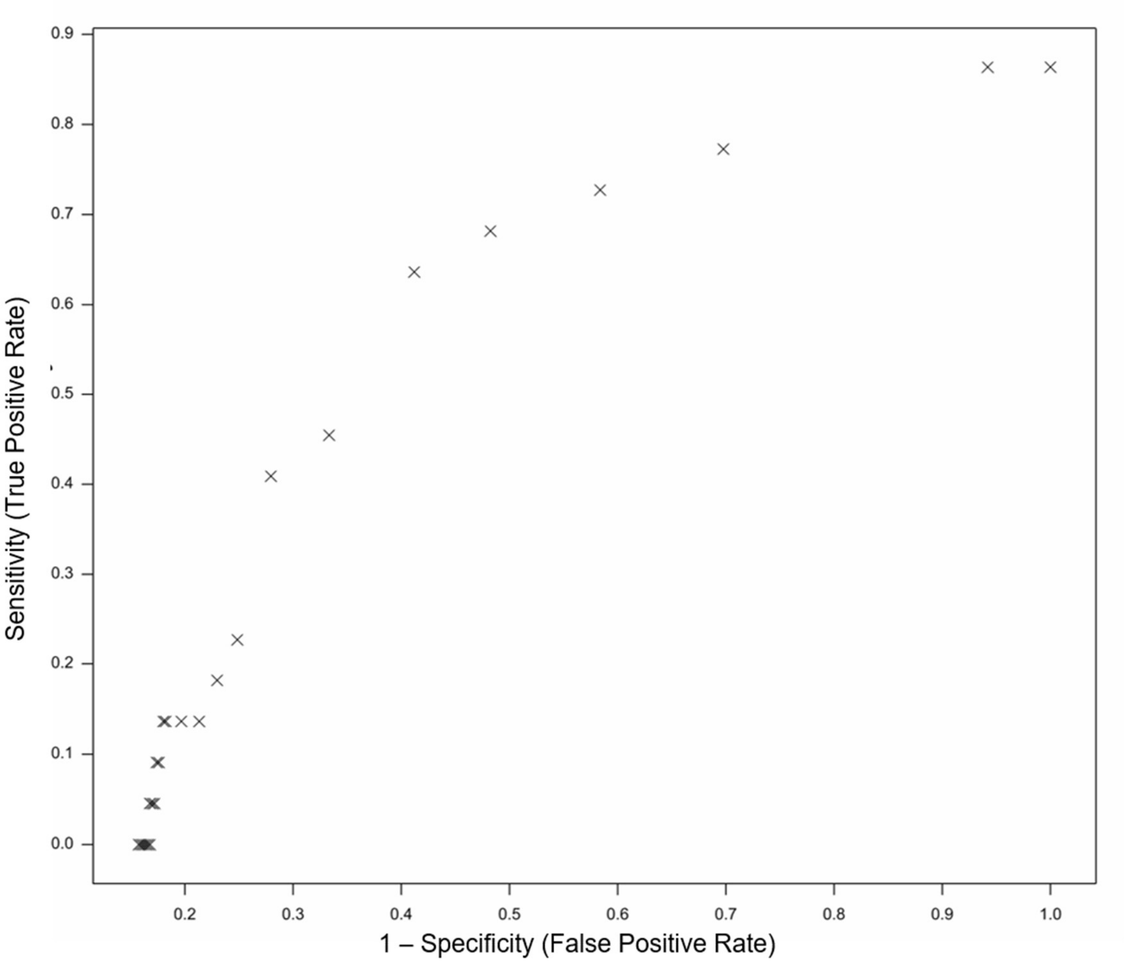


Figure 4.36: ROC Curve based on thresholds of proportions of cow-lactations with metabolic disorders in early lactation estimated from GLMM (Optimum sensitivity = 0.59, optimum specificity = 0.65)

(iii) Subclinical mastitis

Although there were no significant results at the single variable stage (p<0.05), a multivariate model using candidate indicators significant at p<0.1 was constructed for subclinical mastitis (Table 4.9).

Table 4.9: Test statistics for multivariate model including all variables significant at p<0.1 in single variable models for subclinical mastitis

Variable	**Wald-statistic**	**Numerator degrees of freedom**	**p-value (Wald)**
Year of drying	4.88687	1	0.027
Year of calving	0.03824	1	0.844
Yield at drying	5.03985	1	0.024
BW intercept (-60)	1.28351	1	0.257

Year of drying (p=0.027) and yield at drying (p=0.024) were both significant when included in the multivariate model for subclinical mastitis. Year of calving is very highly correlated with year of drying and therefore when added to the model after year of calving, it is insignificant. Despite these significant effects, there is no discrimination between cows that remained healthy and those that developed subclinical mastitis, meaning that the explanatory power of the model is very low (Figure 4.37, Figure 4.38 & Figure 4.39).

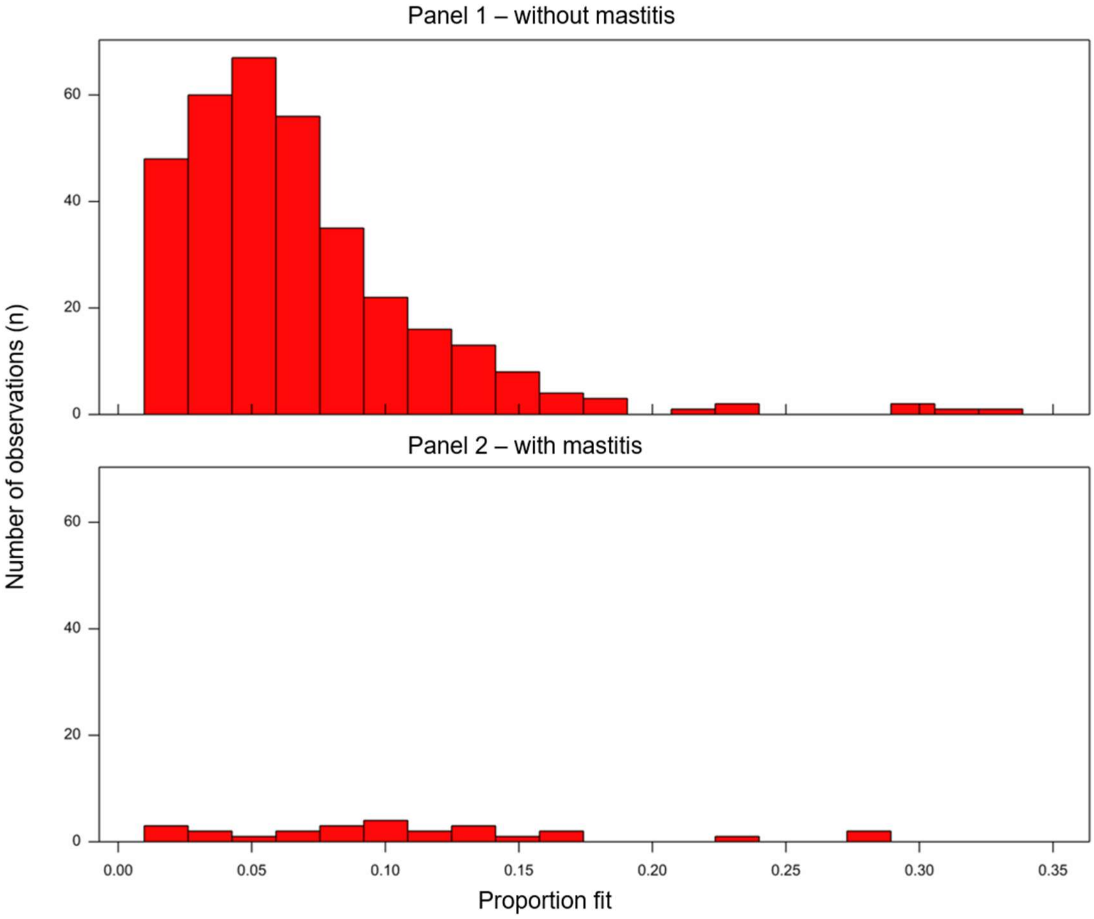


Figure 4.37: Histograms of proportion of cow-lactations with subclinical mastitis estimated from multivariate GLMM for cow-lactations with (Yes) and without (No) subclinical mastitis

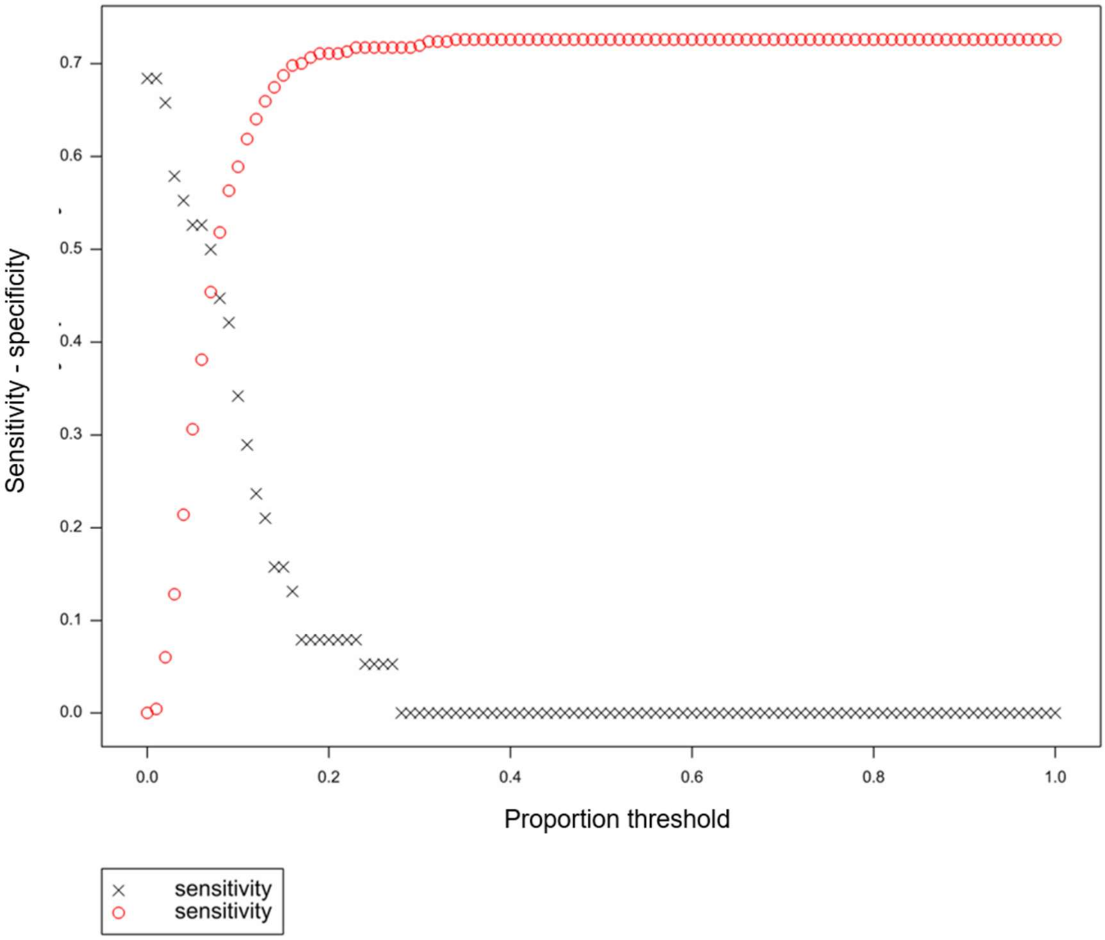


Figure 4.38: Sensitivity and specificity based on thresholds of proportion of cow-lactations with subclinical mastitis in early lactation estimated from GLMM

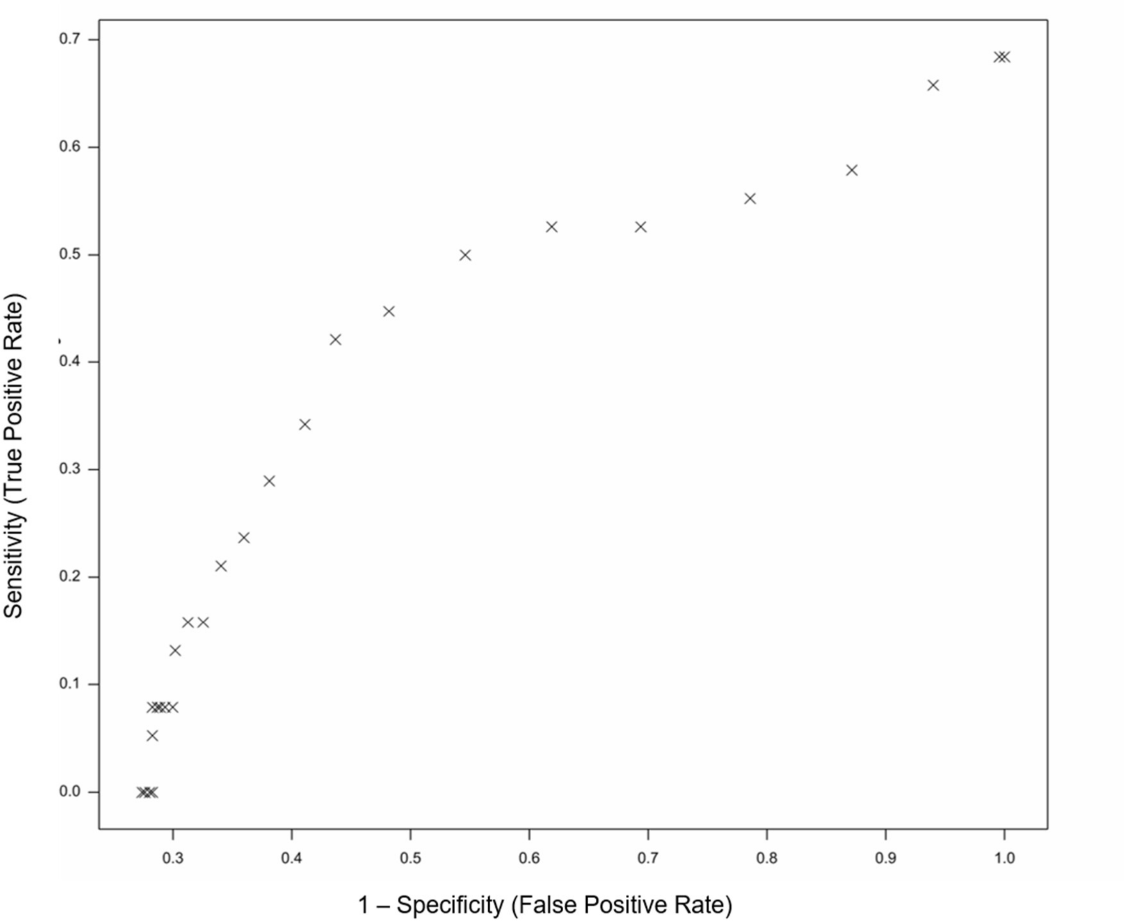


Figure 4.39: ROC Curve based on thresholds of proportions of cow-lactations with subclinical mastitis in early lactation estimated from GLMM (Optimum sensitivity = 0.50, optimum specificity = 0.45)

(iv) All disease

Genetic line (control vs. select), the intercept of body weight at calving calculated from linear regression, change in body energy content over the dry period and dry period length are all significant in the multivariate model for all disease. More variables are found to be significant in this model as all diseases are combined and the comparison is between cows with no clinical disease and cows with any of the diseases (reproductive disorders, mastitis or metabolic disorders). Adjusting for genetic merit and parity mean that several of the other candidate variables lose their significance which was detected in single variable models. Despite 4 of the explanatory variables being significant, the explanatory power of this model is still poor due to wide and large overlaps between the distributions of cows with disease and those without disease (Figure 4.40). Therefore, the sensitivity and specificity of the model is poor (Figure 4.41 & Figure 4.42).

Table 4.10: Test statistics for multivariate model including all variables significant at p<0.1 in single variable models for all disease

Variable	**Wald-statistic**	**Numerator degrees of freedom**	**p-value (Wald)**
Genetic line	3.12336	1	0.077
Parity	2.52519	1	0.112
BW intercept (calving)	5.50609	1	0.019
BEC change	5.89227	1	0.015
BW at dry off	2.57591	1	0.109
BEC intercept (calving)	0.25199	1	0.616
CS change	0.05994	1	0.807
BEC intercept (-60)	0.17458	1	0.676
LW intercept (-60)	0.04739	1	0.828
BEC at dry off	0.95240	1	0.329
Dry period length	6.14476	1	0.013
LW change	0.58529	1	0.444
LW at calving	0.17886	1	0.672
CS at dry off	0.77594	1	0.378

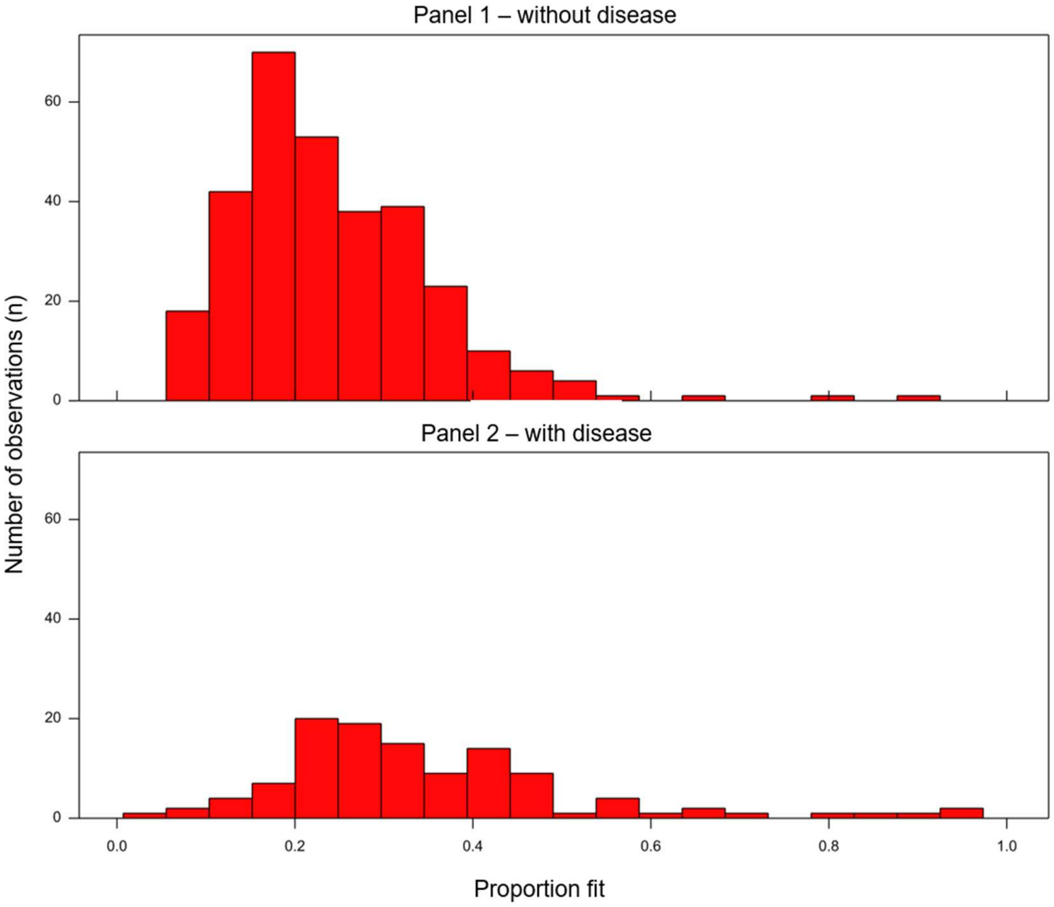


Figure 4.40: Histograms of proportion of cow-lactations with any disease estimated from multivariate GLMM for cow-lactations with disease (Panel 1) and without disease (Panel 2)

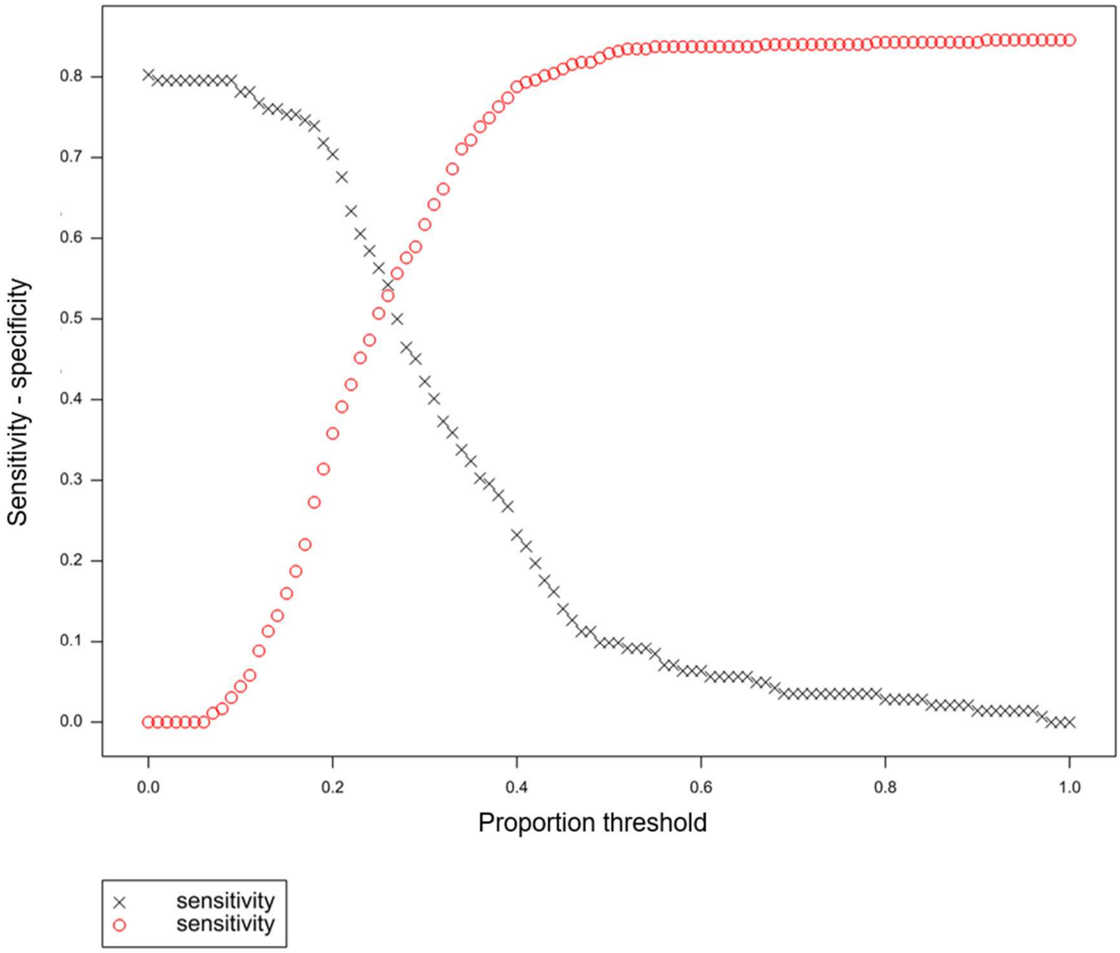


Figure 4.41: Sensitivity and specificity based on thresholds of proportion of cow-lactations with any disease in early lactation estimated from GLMM

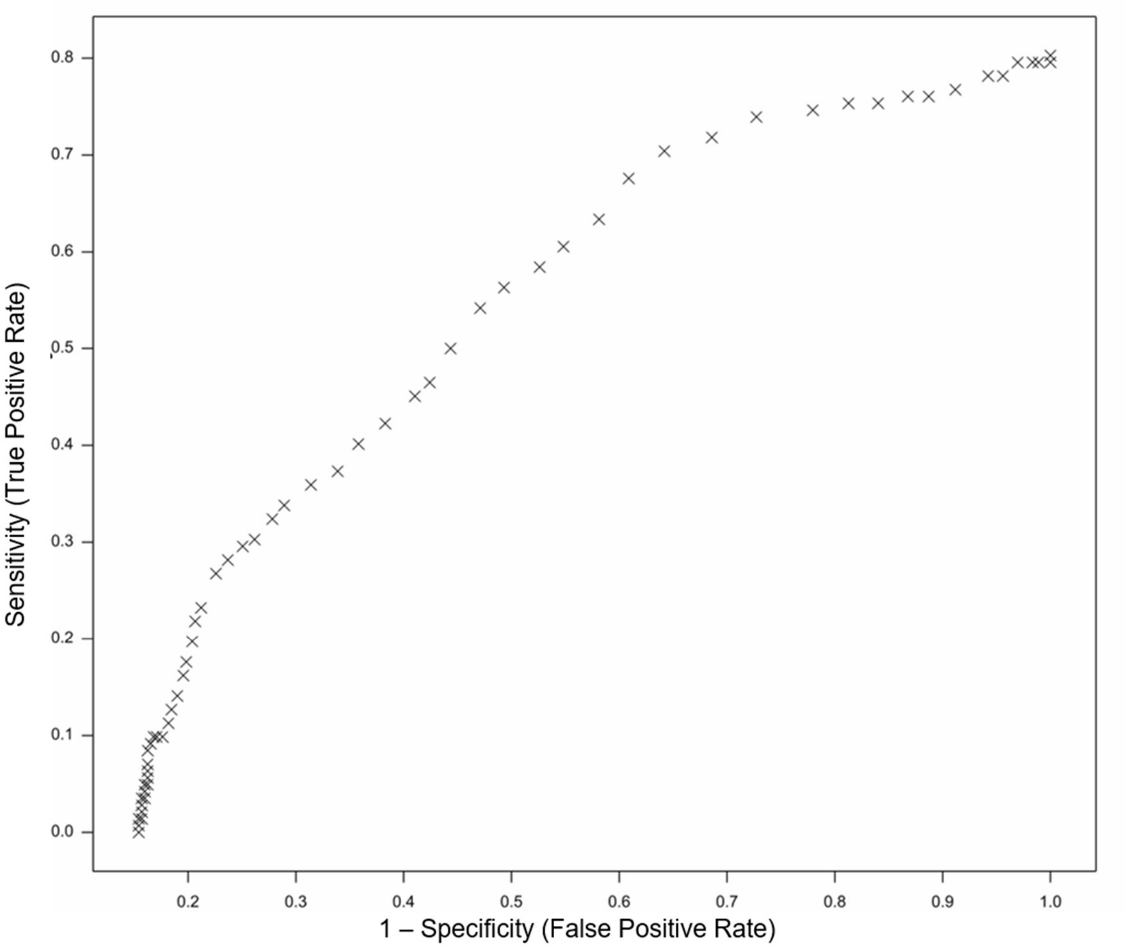


Figure 4.42: ROC Curve based on thresholds of proportions of cow-lactations with any disease in early lactation estimated from GLMM (Optimum sensitivity = 0.59, optimum specificity = 0.65)

4.5 Discussion

In this study, candidate indicators of early lactation disease were investigated as to their predictive ability in identifying cows at risk of disease in early lactation. Although some candidate indicators were reported to have significant effects for some health outcomes e.g. body weight at drying for metabolic disorders, overall the models developed in this study cannot predict disease risk for individual cows due to the large effect of the individual cow, as seen in the large standard errors associated with the variance components of each model. In addition, the high degree of correlation between candidate variables limited the ability to improve the explanatory power of the multivariate models. In general, adding more variables to the model did not explain any more of the variation.

4.5.1 Reviewing disease-specific results

Previous disease history investigated by determining disease incidence in each cow's previous lactation was not found to be significant for any of the health outcomes at the exploratory analysis stage and therefore was not included as a covariate in the GLMM. This finding is somewhat surprising given that much of the literature reports that a previous incidence of disease increases future disease risk. In relation to milk fever, cows that have previously had milk fever have been reported to be 2.2., 2.35 and between 2 and 5 times more likely to develop milk fever again (Erb and Grohn, 1988, Roche and Berry, 2006; Saborio-Montero *et al*., 2017). Similarly, risk of ketosis has been reported to be between 4 and 12 times greater amongst cows with a previous diagnosis of ketosis (Nielsen *et al*., 2005). In the current study, the inclusion of cow as a random effect in the models may to have some extent captured the effect of previous disease history. However, the cow random effect based on previous lactations cannot be used in practice for prediction as it would not be available at the time the prediction is to be made.

(i) Reproductive disorders

Risk of reproductive disorders (retained placenta and metritis) increased as dry period length deviated from 60 days; cows with very extended dry periods were at increased risk of retained placenta and metritis. In a survey of UK dairy farmers, Fujiwara (2018) found that median dry period length was 56 days with less than 6% of farmers reporting dry period lengths of greater than 65 days. Watters *et al*. (2008) did not find an effect of dry period length on disease incidence but the cows in their study were assigned to dry periods of either 55 or 34 days, which are much shorter than those seen in the current study. Interestingly, cows assigned to the short 34-day dry period had significantly lower NEFA concentrations after calving than those assigned to the 55-day dry period (Watters *et al*., 2008). Kok *et al*. (2019) also reported that shortening the dry period improves *postpartum* energy balance. Similarly, studies by van Knegsel *et al*. (2014) and Mayasari *et al*. (2019) report that cows managed to have shorter dry period had less severe negative energy balance in early lactation and lower stress biomarker levels. If the converse of these findings is true, in the context of the current study this may mean that cows with very long dry periods are likely to have higher *postpartum* blood NEFA levels, which in turn increases the risk of early lactation disease.

Candidate indicators related to body energy status (body weight, body condition and body energy content) were also found to be significant as a predictor for reproductive disease in early lactation; as condition score, body weight and body energy content at dry off increase so too does the risk for retained placenta and metritis. Numerous studies in the literature support this finding; Zhang *et al*. (2002) reported that cows with a condition score greater than 3.75 in the late dry period had an increased risk of retained placenta. That condition score at dry off was found to have a significant positive effect on disease risk corroborates the findings of a great deal of the previous work. Shirley (1994), Studer (1998), Heuer *et al*. (1999), Roche and Berry (2006) and Gillund *et al*. (2011) all found high body condition to be associated with higher incidence of disease when compared to cows at optimum body condition. Changes in body weight, condition score and body energy content all had significant negative effects on risk of reproductive disorders – cows that had a negative daily change in these traits had a greater risk of disease than cows which maintained or increased body weight, condition or energy content over the dry period. These results reflect those of Kim and Suh (2003) who found that cows that lost 1-1.5 units of body condition had a higher occurrence of metritis than those that experienced a moderate body condition loss.

(ii) Mastitis

None of the candidate variables were found to be significant for predicting risk of subclinical mastitis in early lactation. A possible explanation for this is that, for the diseases included in the current study, the link between mastitis and the majority of candidate variables which were based on measures of body condition and body weight, is the most tenuous. The adoption of modern milking practices has seen a reduction in the prevalence of contagious organisms however, the most prevalent pathogens now causing mastitis in cows are those that originate from the environment (Oliveira *et al*., 2013). Therefore, as an infectious disease, the presence of pathogens in the environment is likely to be a much more important etiological factor in determining disease risk rather than body energy status of the cow, although body energy status does have an important role in determining immune status. It is widely accepted that high milk yield is a risk factor for future intramammary infection therefore it is somewhat surprising that yield at dry off was not found to be a significant predictor of mastitis in the next lactation. A possible explanation for this might be that the average milk yield at dry off for cows in this study was relatively low at 20.6± 6.49 litres.

(iii) Metabolic disorders

The risk of metabolic conditions was significantly higher amongst older cows (cows moving to lactation 3) compared to younger cows (cows moving to lactation 2). This result corroborates the findings of previous work which has shown that age is a clear risk factor for metabolic conditions, particularly milk fever (Saborio-Montero *et al*., 2017). Roche and Berry (2006) reported that cows in fourth and fifth lactation were 2.30 and 7.43 times more likely to develop milk fever than cows in third lactation. Physiologically, this is likely to be caused by the decreased ability to mobilise skeletal calcium and the decreased production of Vitamin D_3 in older cows (van Mosel *et al*., 1993; Horst *et al*., 1997). Practically this finding supports an enhanced level of management and disease control measures for older cows to limit the expected increase in disease risk. Currently such strategies include the administration of prophylactic calcium to older cows around the time of calving. Select genetic merit cows were found to have an increased risk of all disease compared to control cows. This finding supports previous literature which has associated increased disease risk with higher yielding cows and suggested that selection based solely on production traits has been to blame for the reported increase in production disease (Grohn *et al*., 1989; Fleischer *et al*., 1991; Ostergaard and Grohn, 1999; Rajala-Schultz *et al*., 1999; Ingvartsen *et al*.. 2003).

Furthermore, body weight at dry off, intercepts of body weight at calving and 60 days before calving and the intercept of body energy content at calving (calculated from linear regression) all had significant positive effects on risk of metabolic disease. Cows with a higher body weight at dry off had a significantly greater risk of developing metabolic disease (ketosis, left displaced abomasum, hypomagnesaemia or hypocalcaemia) in the first 30 days of lactation compared to cows with a lower body weight. These results are in accord with other studies which have reported that cows fed a high energy density in the dry period, and consequently gain weight, have an increased incidence of ketosis and displaced abomasum (Cameron *et al*., 1998; McArt *et al*., 2013; Mann *et al*., 2015). Sahar *et al*. (2020) reported similar results which showed that cows that became sick in the 21 days after calving had higher BW than those that remained healthy.

4.5.2 Appraising the methods employed

(i) Exploring distributions

It is recommended that the process of model building should begin by generating univariate descriptive statistics of the data (van Kujik *et al*., 2018). Therefore, in the current study descriptive statistics were calculated for the data, and histograms of data from each variable were plotted to check for normality, skewness and outliers (see Figure 4.5 for example). This is an essential means by which to identify any errors within the data and to gain an understanding of the data e.g. are they normally distributed, are there any outliers, are the outliers due to data errors or do they represent a true value? (van Kujik *et al*., 2018; Giorgi *et al*., 2021). This phase is important in determining whether any of the raw data should undergo transformation to allow better performance in the model developed and for selection of the most appropriate statistical methods. In the current study, all continuous variables selected for use as candidate indicators were standardised to assist model fitting. In the current study, it was decided to retain outliers in the dataset for analysis; although this may have weakened the power of the statistical models developed, from a physiological standpoint it seemed plausible that cows at the extremes of the distributions for many of the candidate indicator variables may have been those cows which went on to develop disease e.g. those with very long dry periods and those with very low or very high body weights. The understanding of the data gained by examining them in this way played a key role in all future stages of the analysis.

(ii) Understanding interrelationships

Correlation between covariates and between covariates and factors was tested to gain an understanding of how each potential predictor related to every other predictor, or ‘nuisance’ variable e.g. parity, month of dry off/calving, genetic line and diet type. Knowing the bivariate relationships among variables gives insights into why certain variables lose significance when added into multivariate models and allows for the detection of over-fitting (Giorgi *et al*., 2021). A certain degree of structural multicollinearity was inevitable in the current study as body energy content was calculated using body weight and body condition score data, which were also used on their own as candidate indicators. For practical reasons, understanding the relationships between candidate indicators was important; where candidate indicators measured at dry off were correlated to those at calving, it would be beneficial to select those measured at dry off for inclusion of the model as the prediction of disease incidence could occur further in advance of the event and therefore preventative actions could be taken.

(iii) Generalised linear mixed modelling

The data set used in this study, like many biological datasets, includes binary data and as such does not have random variation that follows a normal distribution. This precludes standard least square regression techniques as a method of analysis and necessitated the use of GLMM. In GLMM, the ‘value’ of the response measure does not have to equal a linear sum of the fixed and random effects, but will be related to the linear sum by a link function; in the current study a logit link was used which converts covariates to the probability scale. A further key benefit of the GLMM method is its ability to deal with data with non-normal distributions, meaning the binary coding of disease events (present/absent) can be used.

Each candidate indicator was tested in the single variable models as a fixed effect. However, it is not useful for getting predictions to have the fixed effects of genetic line or diet type included in any model unless all commercial dairy farms can be defined in these terms. Thus, it is beneficial that neither of these covariates remain significant in the multivariate models after adjusting for the other indicator variables. However, it is problematic that there is a significant effect of year of dry off in the multivariate model for mastitis as for the development of truly predictive models, the inclusion of year as a fixed effect is not useful. A possible solution would be to include year in the model as a random effect, however examining the fixed effect of year suggests there may be a trend which would make this approach invalid. In an ideal world, where candidate indicators demonstrated large significant effects, the effect of both year and system would become significant after adjusting for the candidate indicators as it would be impossible to quantify the effect of either of these traits in a model being used across many herds over an extended period of time .

(iv) Assessing goodness of fit

The discriminative capacity of each of the single variable and multivariate models was assessed by constructing ROC curves and sensitivity and specificity plots which is a standard procedure for assessing model performance (Nemes & Hartel, 2010). The ROC curve is particularly useful as it captures in a single graph the trade-off between sensitivity and specificity over the entire range of probability thresholds (Nemes & Hartel, 2010). As demonstrated in the series of exemplar sensitivity, specificity and ROC plots, the constructed model offers very little information about the response variable class (disease present/disease absent) and the prediction is completely random. This suggests that there are other factors, beside those included in the model, which exert influence on the response variable. In the case of mastitis, somatic cell count in the previous lactation is likely to have affected future mastitis risk and therefore including it in future models may enhance their predictive accuracy. Feed and water intake, alongside activity and behaviour data may also be useful additional variables to include in future models for reproductive and metabolic disorders, although the collection of this information is more difficult.

In the case that a good retrospective fit to the data is achieved, the current study would have extended to examine the feasibility of a decision support system by showing that the current set of data could be used to accurately predict disease risk within another set of data. Ideally, cross-validation would have been performed either within the data set (by segmenting the data set and assigning some as a "training set") or in other herd datasets if they were available. To assess predictive accuracy in the validation data set, the same methods (calculating sensitivity, specificity and drawing ROC curves) would have been employed.

Very few published papers have developed predictive models; most studies have been associative. However, Sahar *et al*. (2020) followed a very similar series of methodological steps as employed in the current study, in their development of a model to predict disease in transition cattle using feed intake and feeding behaviour. Relative to health status, cow data was classified similarly, distributions of continuous variables were investigated and correlations between variables were investigated before a multivariable binary logistic regression model was constructed. For multiparous cows, their model had a sensitivity and specificity of 66.1% and 81.3% in the training dataset and 73.3% and 80.0% in the test data set, respectively (Sahar *et al*., 2020). The approach taken by van Dixhoorn *et al*. (2018) included intensive clinical examinations and blood sampling of 22 cows in the immediate pre- and *post*-calving periods; each aberrant observation was scored '1', with a total deficit score being calculated for each cow on a daily basis. A best subset selection process was used to obtain a prediction model, which was then tested by means of leave one out cross validation, where each observation was used successively as a validation set (van Dixhoorn *et al*., 2018) ; this offers the opportunity to perform validation without the need for retrieving additional data.

4.5.3 Challenges and limitations

The ability to develop successfully predictive models may have been hampered by the low disease incidence within the study population, which gave rise to low numbers available for analysis. Hazards associated with low numbers include low statistical power, inflated false discovery rate and low reproducibility (Button *et al*., 2013). One method employed to mitigate the effect of the small numbers was to create binary variables for each disease so that each cow-lactation with disease 'x' could be compared to all cow-lactations without disease 'x'. However, by combining different diseases in one group, important information may have been lost as there is likely to be some interaction between disease type and fixed effects – the candidate indicator variables. Options to overcome the challenge of small numbers would be to either access datasets from nationwide recording systems or to conduct an experiment, where cows were balanced for parity, management system etc. and disease was artificially induced in a subset of the animals. A benefit of the current approach over the use of large-scale data sets from milk recording schemes is that management system is very well defined and all the animals were subject to the same general management procedures, which would not be the case in an extended dataset.

A further challenge in this study was the large individual cow variance component. The effect of individual cow was included in all models as a random effect to allow for differences between cows and differences between response measures on the same cow. It was important to average over the cow random effect as the individual effect of cow would not be known in practice. As seen in Tables 4.3, 4.4, 4.5 and 4.6 the random effect of cow (as measured by the individual cow variance component) accounted for a large proportion of the reported variation, which indicates that each of the candidate indicators is explaining very little of the of the variation in disease outcome and the model is failing to truly capture the difference between cows relative to disease status. In the future, genomics may offer a method of understanding phenotypic differences (i.e. disease risk) that cannot currently be captured or measured and therefore provide a better understanding of between cow variation.

Although cases of disease in the study herd were confirmed by experienced stockspeople or veterinary surgeon, there is likely to be several cases where disease was not diagnosed. Further, cases of subclinical metabolic and reproductive disorders were not identified and consequently are unknowingly included in the ‘healthy’ population in this study. Hojsgaard and Friggens (2010) argued that it is beneficial to regard degree of infection (of mastitis) as a latent quality, varying continuously from 0 (truly healthy) to 1 (full-blown clinical mastitis). If this principle applies to other diseases, there may be a significant number of cows who did not have “full blown” clinical disease which was detected but that were “sick enough” to have changes relative to the candidate indicator traits. This has important implications for modelling as cows that were “nearly sick” may have been misclassified as healthy.

4.5.3 Future work

The increase in animal monitoring technologies, as outlined in Chapter 1, offers the opportunity to record and collate more accurate data, more often and more easily. Future work in developing methods of identifying sick or at-risk cows should exploit the available technologies as data sources. In addition to offering more accurate and more easily recorded data, the combination of data from various sources to form a “panel of indicators” has proven to be successful; van Dixhoorn *et al*. (2018) aggregated accelerometer, temperature and rumen bolus data. In their review of sensor systems, Rutten *et al*. (2013) reported that there was no integration of data from different systems, which reinforces the need to explore this in future research. Although it does present some challenges, the use of herd surveillance type data from national recording schemes should also be considered for use in future studies, particularly for use in the validation stages of model development.

The successful development of systems to detect the onset of oestrus and calving have largely relied on detecting changes to the normal values for a series of traits e.g. activity, temperature, eating behaviour. Disturbances in daily patterns of eating behaviour, number of steps and variance in ear temperature have been proven to be able to predict disease severity in early lactation (van Dixhoorn *et al*., 2018). If deviations in body weight or condition score could be calculated routinely, this may offer an opportunity to identify cows at risk of disease. Research work is needed to disentangle the causes of variation in weight and condition score – is it genetically or environmentally driven? If this was established, then that which was apportioned to environmental causes may be more likely to be associated with increased disease risk. Friggens (2004) proposed that cows have a genetically driven defended trajectory relative to body fatness, meaning deviations from the trajectory are generally followed by adjustments to regain the trajectory once environmental conditions allow. His work suggests that the drive to attain a genetically determined fatness changes relative to stage of lactation. If this is the case, future work should focus on developing a method to create a personalised baseline of body energy trajectories for individual cows from which to calculate deviations such as those used in activity monitoring for heat detection.

4.6 Conclusion

Although this study did identify some traits which were significantly different between the populations of sick and healthy cows, the approach did not yield successful predictive models for early lactation disease. This was due to the large and uncaptured effect of individual cow, described in the large variance components and associated standard errors. In addition, the high degree of correlation between variables limited the ability to improve the explanatory power of multivariate models. Low disease incidence in the population made this more difficult. This study serves to prove an important point, that explanatory and predictive modelling cannot be conflated. Significant differences in the means of a trait between two populations is a necessary but not sufficient condition for the development of truly predictive models. Future work should rely on the use of data from the many automated tools now available for individual cow monitoring and seek to create personalised baselines from which to calculate deviations to identify at risk cows. Furthermore, the correlations between variables should be thoroughly investigated so as to identify the variable which has the most explanatory power, is the easiest to measure and is available earliest so as to maximise the usefulness of these models as a means of identifying cows at increased risk of disease.

Chapter Five

General Discussion & Conclusions

The overall aim of this project was to investigate the impact that early lactation disease has on dairy cow productivity, and to identify potential indicators of early lactation disease to allow prediction of disease risk amongst freshly calved cows.

5.1 Discussion

(i) Disease and dairy cow productivity

With the exception of Hostens *et al*. (2012), most of the literature which quantifies the effect of disease on milk yield was performed in excess of 20 years ago, when average milk yields were significantly lower than current yields e.g. Deluyker *et al*., 1991, Østergaard *et al*., 1999 and Rajala-Schultz *et al*., 1999). The initial stage of this study provides an up-to-date estimate of losses in milk yield associated with disease, and has shown that disease in early lactation has a significant impact on milk yield and reproductive performance which has the potential to significantly reduce the profitability and efficiency of dairy production systems. Using a typical farmgate milk price of 27ppl (AHDB, 2020) the loss of income from milk associated with the diseases studied range from £136/cow per case to £244/cow per case (Table 5.1). Incidence of these diseases in the current study were 10.9% for subclinical mastitis, 15.9% for reproductive disorders and 3.5% for metabolic disorders. Using these disease incidences, for a 200-cow herd, milk income losses would total more than £11,000 (£2984 from subclinical mastitis, £6765 for reproductive disorders + £1708 from metabolic disease). The increased interval between calving and first service seen in cows with a metabolic disorder also has an economic implication through its potential to affect calving pattern. DairyCo, now known as AHDB Dairy, recommended a 60 to 65-day calving to first service interval and report that a reduction of 10 days in the interval is associated with a 2% reduction in culling (DairyCo, 2008). Esslemont *et al*. (2001) concluded that for a high yielding cow, the cost of one day of delay in conception was £2.48 when conception was delayed from 85 to 115 days *post*-calving. Using the increases in calving interval reported in the current study as a proxy for increase in days to conception and the costs estimated by Esslemont *et al*., the negative effect of disease in fertility is likely to incurs between £29.70 and £49.60

Table 5.1: Economic impact of disease on milk income and calving interval for cases of subclinical mastitis, reproductive disorders and metabolic disorders relative to cows with no clinical disease

		Subclinical mastitis	Reproductive disorders	Metabolic disorders
Relative to cows with no clinical disease	Milk yield difference (litres)	-507	-788	-904
	Milk income loss[1] (£)	136.9	212.7	244.1
	Increase in calving interval (days)	+12	+13	20
	Cost of increased calving interval [2](£)	29.7	32.2	49.6
	Total cost of milk income loss + extended calving interval per case	166.6	245.0	293.7
	Disease incidence[3] (%)	11%	16%	4%
	Number of cases for 200 cow herd (n)	21.8	31.8	7
	Total cost (£)	3632.9	7791.0	2055.7

[1] Using a current typical farmgate milk price of 27ppl (AHDB, 2020)

[2] Using £2.48/day cost of increase in calving interval (Esslemont *et al.*, 2001)

[3] Using disease incidence reported in the current study

depending on the disease (Table 5.1). The costs reported in Table 5.1 do not account for the increased culling rates seen in cows with disease, which increases the cost of replacement heifers nor does it include the treatment costs. There is a lack of up-to-date published literature detailing the costs of disease, with the most comprehensive analysis being that of Kossaibati and Esslemont (1997) which uses 1995 costs. The economic impacts associated with disease reinforce the importance of reducing disease incidence to protect both profitability of dairy production systems and animal welfare.

(ii) Milk yield and disease risk

An important finding from Chapters 1 & 2 is that the use of milk yield to identify cows at risk of disease is complicated. This complexity arises from the fact that overall level of milk production is associated with changes to disease risk, while short-term changes in milk yield are often symptomatic of individual disease events. Although short-term changes in milk yield can be effectively used to identify cows suffering from disease, the early identification of cows that are at risk of developing disease in the future is complicated by this cause-effect relationship. Using mastitis as an example, it has been understood for decades that higher yielding cows tend to have a higher incidence of intramammary infections (Schukken *et al*., 1990), while simultaneously it is also well documented that mastitis reduces the quantity and quality of milk produced (Barielle *et al*., 2003; Enger, 2019). Although the elucidation of the cause – effect relationship between milk yield and disease risk is beyond the scope of the current study, the finding that the genetic indexes for milk yield were highest in cows with a diagnosis of subclinical mastitis compared to healthy cows was interesting. The most plausible explanation for this result is that cows diagnosed with subclinical mastitis formed part of a higher yielding cohort within the herd, and consequently had an increased prior risk of developing intramammary infections, rather than mastitis "causing" an increase in milk yield. This finding is consistent with that of Grohn *et al*. (1995) who reported that cows with mastitis are often higher yielding and yield more milk even after having contracted the disease, compared to their healthy herdmates. A further complicating factor in the relationships between milk yield and disease was detailed by Detilleux *et al*. (1997) who found that milk yield losses associated with an incidence of LDA were greatest among the highest yielding cows. For the modern dairy producer, these results suggest that enhanced disease prevention measures are necessary for very high yielding cows due to their inherently increased risk of disease. For the purposes of developing precision livestock farming tools, these results provide support for the hypothesis that milk yield itself is not useful for identifying cows at risk of disease – particularly for early lactation disease where the additional challenge is that milk yield data is not available for the dry period which immediately precedes the time of highest disease incidence. Therefore, it is necessary to use alternative measures of health status in the dry period which are associated with early lactation disease risk and can feasibly be recorded on commercial dairy farms.

(iii) Identifying candidate indicators of disease and developing predictive models

The objective of the second study in this project was to identify candidate measures of physiology and productivity from the end of lactation and dry period which could be used to distinguish between healthy and non-healthy cows, with a view to the future inclusion of these candidate measures in predictive models. Candidate variables were selected carefully based on biological plausibility and previous research as it is acknowledged that testing too many candidate variables may lead to type I errors (Sainani *et al.*, 2014). The explanatory model which was developed found associations between measures of body energy content, body weight, body condition score and milk yield at dry off and future disease status in some cases. Cows that developed reproductive disorders had a significantly different pattern of change in body energy content in the first two weeks of the dry period compared to cows that remained healthy. Practically, this highlights the importance of end of lactation and far-off dry cow management on future health status and emphasises the relevance of studying the whole dry period when considering disease risk in the following lactation. The end of lactation should be considered as a critical time point within the lactation-gestation cycle and cows should be managed in a manner which does not increase their risk of early lactation disease. Although most cows will naturally recover some body condition in the early dry period, the majority of manipulation of body condition should be performed in late lactation to avoid the need for extreme diets in the dry period, when calf and udder development may be affected (Scottish Agricultural College, 2007). While average milk yields continue to increase, adoption of practices to facilitate abrupt dry-off without impairing animal welfare should be encouraged e.g. the administration of cabergoline rather than feed and water restriction. Optimal feed space should be provided to ensure that all cows have free access to feedstuff; Huzzey *et al.*, (2012) found that overstocking cubicles (200%) and providing 34.5cm of feed bunk space increased blood NEFA levels compared to 100% stocking density and 68.5cm of feed bunk space. For cows most at risk of developing disease, i.e. those in the very early and very late dry periods, sufficient feed space (>76cm per cow) is necessary to ensure cows can maximise DMI and therefore make a smooth transition from and to lactation (DeVries, 2019). The effects of the stress associated with dry-off and the significant management changes cows are subject to at this stage of lactation on production and health measures warrant further study.

Having identified significant associations between several candidate variables and health outcomes in early lactation, the final stage of this study was to assess the ability of these variables to form a predictive model. However, despite the significant associations reported in Chapter 3, it was not possible to develop any models for predicting the probability of disease in early lactation with an acceptable level of accuracy. Although significant differences exist between the means of candidate variables for cows that remained healthy and those that developed disease, large ranges in the variable measurements within each health group meant that there was not clear discrimination between the populations.

Furthermore, the fact that accurate predictive models could not be developed despite significant associations existing between the outcomes and candidate variables, highlights an important point – explanatory and predictive modelling cannot be conflated. Explanatory models seek to identify individual risk factors (candidate variables) that are associated with the outcome (health/disease status) and to identify confounding variables (those that are linked to both the risk factors and the outcomes) (Sainani, 2014). In contrast, predictive models aim to “accurately estimate the probability that a disease is present or that a future event will occur” (Sainani *et al.*, 2014). At all stages of the model building process from study design and data collection to assessing model accuracy, the approach is different for explanatory and predictive modelling; relative to sample size, predicting accurately in a prospective manner requires more data than retrospective analysis, due to the extra statistical uncertainty (Shmueli, 2010). Additionally, the process of variable selection for each type of modelling should also be approached differently. In predictive modelling, there is no need to understand the underlying "causal structure" or biological plausibility of the variable as a predictor, but rather variables ought to be selected based on the quality of association between predictor and response, data quality and the ex-ante availability (the availability of predictor data at the time of prediction (Shmueli, 2010)).

Relative to transition cow disease, the majority of published studies use explanatory modelling to investigate possible causal pathways between the variables, and very few studies using predictive modelling exist, with the exception of Vergara *et al*. (2014) and a series of studies by Wisnieski *et al*. (2019a; 2019b; 2019c; 2019d). For *multiparous* cows, Vergara *et al*. (2014) used parity, calving abnormality, previous lactation milk yield, *prepartum* locomotion score and a variety of interactions between these variables in a predictive model for treatment or removal from the herd within 30 days of calving which had a sensitivity of 60%. Due to the lack of information available for *primiparous* cows, only age at calving and calving abnormality were included in the predictive model for heifers which was only able to achieve 35% sensitivity (Vergara *et al*., 2014). In the case of *multiparous* cows, the model was moderately accurate and used data which is readily available on the majority of commercial dairy farms and does not involve any invasive procedures. In the current study, data was also selected for evaluation as to its availability on commercial farms. Condition score assessment can be performed routinely by farmers without the need for investment in specialist equipment and is therefore, in theory, available on all commercial dairy farms, should the farmer choose to record this information. Automated means of assessing condition score are also available e.g. Song *et al*. (2019). Body weight data is only available where walkover weigh scales are installed. However, the increase in adoption of robotic milking systems means that a growing number of farms can measure body weight accurately and routinely throughout lactation. Recording body weight throughout the dry period still presents a challenge, other than in research herds. Future work should focus on specific types of on-farm data and how they relate to health outcomes.

Biomarker data was used by Wisnieski *et al*. (2019a) to develop predictive models for transition disease risk. Rather than using biomarker data from early lactation, when there is little opportunity for interventions to reduce disease incidence, the researchers in this case measured biomarkers at the point of dry-off. They developed a model based on 3 individual models for each component of metabolic stress (nutrient metabolism, oxidative stress) and achieved impressive predictive ability (area under curve = 0.93) thus indicating that it may be possible to detect cattle at risk of transition disease at the point of dry off (Wisnieski *et al*., 2019a). A major drawback of this approach, however, is the invasive nature of obtaining blood samples to allow for biomarker analysis and the associated cost. Further to this initial study, Wisnieski *et al*. (2019b; 2019c) sought to aggregate data from individual cow-level to group-level by compositional modelling, to predict early lactation disease at dry off. These studies proved proof of concept for inferring group-level disease risk from cow-level data, but the models tended to overestimate disease incidence in groups with low observed disease counts and underestimate disease incidence in groups with high observed disease counts (2019b; 2019c).

(iv) Challenges and limitations of the study

A key limitation throughout all analyses in the current study is the low sample size relative to each disease. The data used in this study was recorded over an extended time period and had the advantage of being sourced from a highly respected research herd which is well-described in the literature as to its genetic composition and management systems (Pryce *et al*., 2001; Ross *et al*., 2014). The management of animal feeding, breeding and health was performed according to standard operating procedures and were regularly assessed throughout, meaning that the generated data was reliable. This type of experimental data is preferred for explanatory modelling (Shmueli, 2010). However, although a significant number of cow-lactations were used (n=482), a key limitation of this study was the low disease incidence. Although the incidence of metabolic conditions was similar to that reported in literature (Whitaker *et al*., 2004), the small number of cases necessitated the grouping of left displaced abomasum, hypocalcaemia, hypomagnesaemia and ketosis. Although mainly associated with early lactation, each of these diseases have multifactorial aetiology and symptoms and therefore grouping them may have caused some association to become lost. This low incidence has implications for the statistical analysis performed and offers an explanation as to why no significant differences in the candidate indicators were found between cows that developed metabolic disease and those that remained healthy. Large standard errors exist for all the measures calculated for the metabolic group which means that in most cases, they are not significantly different from any other health group. Use of population data from national recording programmes would offer the opportunity to significantly increase sample size, however, it has the disadvantage of being pooled from herds of varying breeds, milk yields and management systems where disease diagnosis would not be standardised.

The random effect of cow accounted for a large proportion of the reported variation, which means that the candidate indicators were not adequately capturing the true difference between individual animals relative to future health status. In the case of oestrus detection where very frequent data is available, the algorithms used to process recorded activity data generally compare the individual cow to her individual baseline to identify oestrus e.g. the algorithm proposed by Roelofs *et al*., (2005) measures an increase in the number of steps taken by a cow compared to the number of steps taken in the same time period during the preceding 10 days. Similarly, the methodology employed by Løvendahl and Chagunda (2010) for oestrus detection was based on measuring deviations from reference values, which were calculated on an hourly basis for each cow. The challenge in employing a similar method using body weight and body condition score data is that it is recorded relatively infrequently throughout the dry period. Consequently, within the timeframe of a dry period (eight weeks) it is difficult to determine a baseline from which to measure deviations, especially when changes in body weight and condition occur over an extended period.

A further challenge presented by this study is the fact that management system, parity and calendar year had significant effects in the series of statistical analyses performed. Although this identified some interesting relationships between health outcomes and management system, it presents a challenge when seeking to apply the results of the study to other herds and when seeking to move from explanatory to predictive modelling. Within the confines of this study, each management system is well described and understood, but for predictive models to be useful in practice, they must be applicable across management systems and farms (Wisnieski *et al*., 2019d). This can only be achieved when a model is developed with a sufficient number of farms or systems within the sample to allow for farm to be used as a clustering variable and represented as a random effect (Wisnieski *et al*., 2019d).

(v) Looking to the future – future work and alternative approaches

The rapid adoption of AMS and associated technologies continues to create a growing opportunity for accurate data recording and monitoring on an increasing number of commercial dairy farms. On a farm management level, this represents an opportunity to manage cows on an individual basis with tailored approaches according to genetic potential, previous performance and health and nutritional status. For the development of tools to monitor, detect or predict disease, the volume of data generated by new and emerging technologies offers the opportunity to include and aggregate cow-level data from a number of sources e.g. AMS, accelerometers, breath sensors, to improve the predictive accuracy of any such models by more accurately capturing the status of an individual cow at any given time. Adoption of the approach taken in the development of oestrus detection aids i.e. establishing a ‘baseline’ from which to calculate deviations, is essential in overcoming the challenge presented by the large and unexplained between cow variation seen in the current study. In the context of monitoring body energy status, in future work, it may be useful to use individual cow data from the previous lactation or herd-level parameters to determine an “expected” or “ideal” pattern of body weight and condition change in the dry period from which to calculate deviations and act as a proxy baseline. The increasing adoption of genomic selection offers a means to better understanding phenotypic differences in functional traits, and potentially disease resistance, which cannot currently be captured and could also play a role in establishing an “expected” or “ideal” baseline for a number of traits.

It is essential that future research in the field of dairy cow disease monitoring moves beyond explanatory modelling and identifying risk factors to developing truly predictive models. The vast majority of work in this field cannot be directly used for the development of monitoring tools, as was demonstrated in the current study where significant differences in traits between ‘sick’ and ‘healthy’ cows were not sufficient for the development of accurate predictive models.

From a methodological perspective, the use of machine learning should be explored in future studies. It offers an opportunity to effectively process the large volumes of data required to generate and develop truly predictive models. This technique relies on the theory that computers find patterns and relationships in data without being manually programmed to do so, which allows them to develop a model which can then be used for prediction (Hidalgo *et al*., 2018). This approach has been tested in the prediction of subclinical (Ebrahimi *et al*., 2018) and clinical mastitis (Hyde *et al*., 2020), insemination outcomes (Fenlon *et al*., 2016) and lameness (Taneja *et al*., 2020).

The breeding goals of dairy herds continue to be broadened by the inclusion of an increasing number of functional traits in selection indices, including feed efficiency. The interest in breeding for functionality is likely to continue as advances in technology provide better indicator traits for fertility, mastitis, metabolism and energy efficiency (Egger-Danner *et al*., 2015). The adoption of genomic selection means that the rate of improvement in functional traits will increase. Alongside improvements in management and the development of new monitoring tools, it offers the opportunity to reduce disease incidence and severity and thereby improve animal welfare and farm profitability.

5.2 Conclusion

Disease in early lactation represents an animal welfare challenge for dairy cows as well as negatively impacting on dairy herd profitability through reduced milk yield and impaired reproductive performance. Associations exist between physiological measures and production parameters recorded in late lactation and throughout the dry period with disease incidence in early lactation. The main candidate indicators identified as being different between cows of different health status *post*-calving relate to change in body weight, body condition score and body energy content throughout the dry period. However, in the current study it was not possible to develop predictive models using these candidate variables with a satisfactory level of accuracy. Future work should focus on developing predictive models for early lactation disease, using appropriate statistical techniques and progressing from the many explanatory studies in the current literature. Frequent measures of body condition score and body weight can easily be used to calculate energy status, which is associated with degree of physiological stress and disease risk. Targeted monitoring of cows at the start and end of the dry period should be conducted, as both of these transitions represent a period of intense physiological challenge for the dairy cow. The key findings of this current study can be summarised as follows.

1. Cows with no clinical disease during the first 30 days of lactation produced significantly more milk than cows with reproductive or metabolic disorders. Total 305-day yields were reduced in cows with reproductive and metabolic disorders compared to cows without disease by 788 and 908 litres respectively. Peak yield was significantly reduced in cows with subclinical mastitis (-1.9 litres), reproductive disorders (-2.5 litres) and metabolic disease (-4.2 litres) relative to healthy cows
2. Cows with subclinical mastitis in early lactation, as indicated by elevated somatic cell count, had reduced 305-day milk yields when compared to cows with no clinical disease despite having a higher genetic index for milk production. This effect was statistically significant when data from poorly fitting lactation curves was excluded from the analysis.
3. The resumption of normal oestrus activity was significantly delayed amongst cows that had metabolic disease in early lactation; the average days to first observed

heat was 95 days in milk which was 27 days later than cows with no clinical disease.

4. Conception to first service rates were not different between cows with or without disease, however, the 100 day in-calf rate was significantly different between groups. Cows with reproductive disorders had a 100 day in-calf rate of 25% which was substantially less than that of cows with no clinical disease.
5. The main cause for culling was infertility, which accounted for 25.4% of culls. Eight five % of cows with reproductive disorders were eventually culled for fertility related issues – a significantly greater proportion than were culled for fertility in any of the other disease groups.
6. Cull rate, at the end of the study period, was significantly higher amongst cows with subclinical disease compared to all other cows.
7. Changes in body weight, body condition score and body energy content throughout the dry period were significantly affected by production system. Regardless of genetic merit, cows fed a low forage lactation diet mobilised body reserves in the dry period whereas cows fed a high forage lactation diet gained body reserves.
8. Cows that went on to develop reproductive disorders in early lactation had a significantly different pattern of body condition score, body weight and body energy change in the dry period compared to cows that did not develop reproductive disease. They lost, on average, 18.26 MJ/day for the first 15 days of the dry period. Over the whole dry period they lost more than twice the body energy content of cows that remained healthy.
9. Milk yield at dry off was significantly higher amongst cows that went on to develop subclinical mastitis in the first 30 days of the following lactation compared to cows that developed reproductive or metabolic disorders.
10. Although several of the candidate indicators were identified as being significantly different between ‘healthy’ and ‘sick’ cows and showed potential as predictors for disease, it was not possible to build accurate predictive models for disease. The primary reason for this is the fact that despite significant differences in the means of the indicator traits between the 2 sub-populations, the range of observed values within each population was large and overlapping.
11. The economic cost of disease is substantial. Using the incidence of subclinical mastitis and reproductive and metabolic disorders reported in this study, the

annual cost of impaired fertility and the reduction in milk income associated with disease is estimated to be greater than £13,000 for a 200-cow herd. This does not include treatments costs or account for the higher culling rate seen in cows with disease. Thus, a reduction in disease incidence and severity facilitated by better monitoring tools would be of benefit in improving the profitability of dairy production as well as in protecting animal welfare

12. The development of tools for the automated detection of disease is an important step in reducing the negative impact that disease has on profitability and animal welfare. In order that accurate and commercially useful tools for prediction of disease risk are developed, it is essential that future research progresses from associative research designed to identify risk factors for disease. Explanatory and predictive modelling cannot be conflated and as such, much of the current work in this field cannot be directly used to develop monitoring tools.

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