Analysis of effects of negative energy balance in dairy animals using data from three regions of New Zealand's North Island.

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Summary.

New Zealand dairy industry is among the successful in the world making dairy products its largest export earner. The country receives rainfall throughout the year and over 90% of farms practice spring calving which coincides with peak pasture growth. Milk solids, average herd size as well as total dairy cow population have been increasing in recent years. Most of the dairy cows are located in the North Island of the country, accounting for 62.9% of national totals with 30% of these concentrated in the Waikato region. The herd improvement program is carried out where milk herd test are conducted and analyses for various parameters like SCC, milk solids and reproduction performances are carried out which are later used in decision making. Friesian is the most predominant breed in New Zealand making up 47% of the population, followed by 36% cross-breeds, mostly Friesian x Jersey, 15% Jersey, 2% Ayrshire and a small proportion of other breeds.

Energy balance (EB) is an important parameter in dairy cows which is defined as the difference between energy intake from feed and energy required for body maintenance, production and gestation. When animals are in NEB, they undergo several physiological and metabolic changes which may predispose them to several negative effects like poor reproduction performance and poor immunity. Reproductive diseases like mastitis, metritis, and retained placenta among others have been associated with NEB in high intensive dairy systems of the Northern hemisphere. NEB is common in early postpartum when cows fail to meet the energy requirements of milk production, and it is mostly pronounced in high producers. Insulin- growth factor and related metabolites are affected in this state which eventually leads to poor conception rates.

Data from 535 dairy herds (129,947 cows) of three regions of New Zealand's North Island (Bay of Plenty, Northland and Waikato), were used in this study to investigate the effect of NEB on production, udder health and reproductive performance of dairy cows. NEB was defined as an elevation of FPR in milk of 1.5 and above for the first herd milk test. As a proxy for udder health, "subclinical mastitis" was defined as somatic cell counts (SCC) >250,000 Two linear mixed (FPCM and logSCC) and three mixed logistic models (subclinical mastitis, pregnancy and culling rates) with herd as a random effect were performed with NEB being the primary predictor of interest . Survival analysis using a cox regression model fitted with a gamma-frailty term for herd was done to assess PSM to conception interval.

NEB was positively associated with milk production (+3kg solid adjusted milk per day) and a significant predictor for subclinical mastitis, pregnancy and culling rates, but did not affect the interval from planned start of mating to conception. Cows with NEB had on average 1.2% lower

pregnancy rates accounting for 0.12% lower pregnancy in the population. Subclinical mastitis was also associated with reduced pregnancy (RR = 0.959) accounting for 0.46% of the final in-calf rate at population level. Cows with NEB had on average 3,700 higher milk SCC/ml. There was a complex relationship among factors since NEB increased subclinical mastitis incidence which in turn reduced pregnancy rates. Farms with inadequate transition cow management may experience grave NEB problems with a possibly strong economic impact on net returns from dairy farming. Winter feed planning, availability of supplement feed and other preventive measures (e.g. rumensin) are available for prevention.

Dedications

To my wife Eustacia, you were patient with me for the two years I was away from the family and guided the children ably during this time. Chipego and Chileleko, you always brought delight to me even though I was away from you at a critical stage in your lives. To I dedicate this work to you three people and thanking God for the guidance up to the end.

Acknowledgements

Abbreviations

AI	Artificial insemination
DIM	Days in milk
EB	Energy balance
FPCM	Fat-Protein corrected milk
FPR	Fat protein ratio
ICC	Intra-class correlation
LIC	Livestock Improvement COOPERATION.
logSCC	Natural log of somatic cell count
MPI	Ministry of Primary Industry
NEB	Negative energy balance
PSM	Planned Start of Mating
SCC	Somatic cell count

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1 Chapter 1 - Literature Review

1.1 Introduction

This literature review will highlight published literature on the New Zealand dairy industry. It will highlight both the management system used and the performance of the industry at world level in terms of international trade of dairy products. Some of the reasons that make part of the success story for the dairy industry in New Zealand will be evaluated. The literature reveal is also about some of factors that bring limitations in the dairy industry in general such as production, metabolic and physiological changes, which may eventually lead to poor reproduction performance, low production and/or disease occurrence. Effects of energy balance in dairy cows will be revealed in relation to production, reproduction and risk of disease.

1.2 Dairy farming in New Zealand

The New Zealand dairy industry is one of the most successful in the world as evidenced by ranking in eighth position in 2009 by producing approximately 30% of the world dairy trade, making dairy produce the country's single largest export earner (Bencheva and Garnevska.2011). New Zealand has a natural advantage for agriculture especially dairy farming where animals are kept out-door on pasture all year round (de Klein *et al.*2006) in a mild temperate climate not requiring cow housing thus reducing milk production costs. The country receives rainfall throughout the year with usually a mild winter weather compared to European or North-American climates thus giving a suitable environment for pasture growth. Other than Central Otago, pasture growth occurs all year round throughout the country. Dairy cows are bred to convert pasture forage into milk which contributes significantly to the human diet and economic growth at farmer level and eventually national level.

Over 90% of all New Zealand dairy farms operate spring-calving in the months of July to September at which time dry matter (DM) from pasture is at the peak (Verkerk.2003). The growth of pasture has been associated with the amount of milk solids processed at national level as shown in Figure 1.1 for the Central Waikato region. It would therefore make logical sense to target calving at times of the year when pasture growth is highest so as obtain maximum profits from milk. Pasture growth starts to increase around August and reaches its peak between September and October and then drops in summer. It then remains almost constant following the autumn rains in March to April, a trend that is similar to amount of milk solids processed. Therefore, farmers target their cows to calve in early spring and have their peak lactation in October to December so as to coincide with abundant pasture this time of the year. Nearly all farms use artificial insemination (AI) subsequent to a 'planned start of mating' (PSM) (Alawneh *et al.*2012). The mating period (MP) starts 2 to 3 months after peak calving and lasts about 10 to 12 weeks. In the later mating period, AI is stopped and bulls mate with cows that did not conceive to AI. Failing to successfully breed and conceive within the 12 week period would lead to late calving in the subsequent season. At mating, animals are expected to be in good body condition so as to be able to conceive following a maximum of 2-3 services. Cows are dried off two months before planned start of calving at the same time or in subsequent mobs where it involves large herds (Rognant.1998).

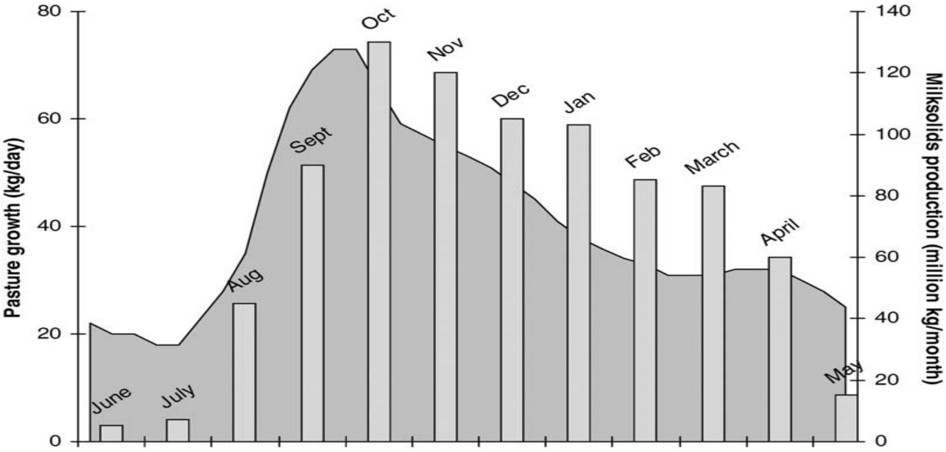

Figure 1.1 Typical pattern of annual pasture growth in Central Waikato, New Zealand (grey area) and monthly national milk solids production (columns). .

New Zealand exports several dairy products which include milk, cream (both concentrated and non-concentrated), buttermilk, cheese, whey and curd (AREN.2008). The largest product category is milk powder, particularly whole and skim milk power, followed by cheese, curd, butter and non-concentrated products. Milk production in the New Zealand dairy industry has increased in recent years. Figures from 12 seasons (DairyNZ.2012) prior to 2011/12 showed that processed milk and processed milk solids increased in recent years by about 60% since 2000/1 (Table 1.1). A total of 19,129 million litres of milk was processed during the 2011/12 season translating to 1.685 million kg of milk solids, that is 954,000 kg milk fat and 731,000 kg milk protein. This was an increase of about 6 million litres compared to 13,606 million litres of milk in 2001/02 season which resulted in 1,152 million kg milk solids, i.e. 657,000 kg milk fat and 495,000 kg milk protein (DairyNZ.2012). New Zealand dairy farmers are paid on basis of milk solids. Rewards are given for fat and protein content but this penalises high volume producing cows that have low fat and protein but high lactose percentage in their milk (Verkerk.2003). From the figures shown in Table 1.1, it could be projected that the dairy industry is continuing to grow.

Table 1.1 Milk processed and milk solids for 12 seasons.

Season	Milk processed (*million litres*)	Milk fat processed (*million kgs.)*	Protein processed (*million kgs*.)	Milk solids processed (*million kgs*.)
2000/01	12,925	626	470	1,096
2001/02	13,607	657	495	1,152
2002/03	13,906	676	515	1,191
2003/04	14,599	716	538	1,254
2004/05	14,103	694	519	1,213
2005/06	14,702	724	543	1,267
2006/07	15,134	750	566	1,316
2007/08	14,745	722	548	1,270
2008/09	16,044	791	602	1,393
2009/10	16,483	817	622	1,438
2010/11	17,339	859	654	1,513
2011/12	19,129	954	731	1,685

The trend in the number of herds, total cow population and average herd size at national level for the past 37 seasons from 1974/75 to 2011/12 is shown in Figure 1.2. The average herd size has gradually and consistently increased from the 1974/75 to the 2011/12 season. The total cow population has equally been on a consistent increase whereas the number of herds declined until the 2008/9 season when a small increase of 182 was observed compared to the previous season of 2007/8, from 11,436 herds to 11,618 herds. Due to a sudden, substantial rise of pay-outs for milk, herd numbers started increasing again from 2008/9 season onwards and stood at 11,798 in 2011/12 season with an average herd size of 393 animals. By that time, the average herd size had increased more than threefold compared to 1974/75 while the total cow population had doubled to 4.6 million during the same period.

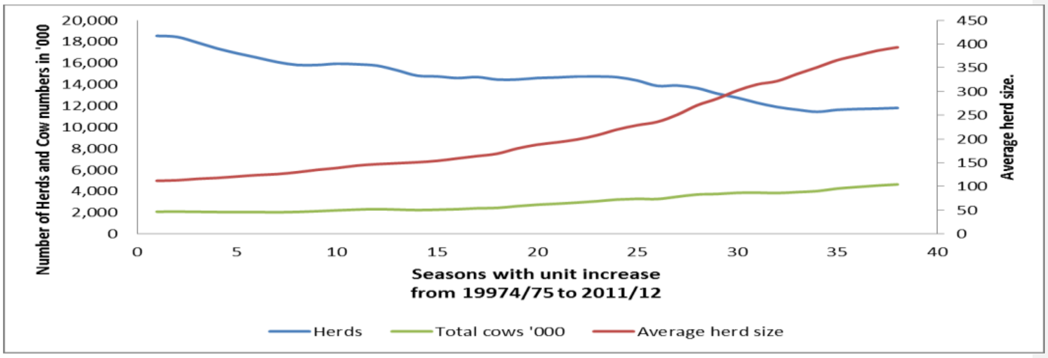

Figure 1.2 Trend in the number of herds, number of cows in thousands and average herd size for the last 37 seasons. Each unit increase on the x-axis represents a unit increase in season. (Source: New Zealand Dairy Statistics for 2011/12).

The New Zealand dairy industry success could be attributed to, among other things, the historic commitment of farmers to generate and record information on the efficiency with which dairy cows produce milk, and using this information for selective breeding to improve the genetic makeup and eventually productivity of their herds (MPI.2012). The herd improvement program for the New Zealand dairy industry has been of importance in improving the efficiency of milk production by dairy cows. The livestock Improvement Corporation Ltd (LIC), which is a dairy farmer owned cooperative, performs activities aimed at herd performance improvement which include herd testing, artificial breeding and database management. The herd testing service involves the collection of individual composite milk samples from each milking cow in the herd which are later analysed for composition. The other data collected during herd testing include information on mating, calving and animal health. The total milk volume produced on the day of testing is also recorded and each sample is tested for milk fat, milk protein, and somatic cells count (SCC).

Herd testing information provides a record of the production of each individual animal in the herd which allows the farmer to make decisions about e.g. mating, treatment plans for diseases, drying off for low producers in advanced lactation stage, culling and selection for replacements (MPI.2012). The information also helps the farmer to identify high performing animals in the herd and select them for breeding. LIC collects and analyses composite milk from each lactating cow at times nominated by the farmer during the season and returns a range of comprehensive animal performance reports which support strategic decision making on farm.

Most of the dairy cows (62%) in New Zealand as of 2012 were in the North Island while 37.1% were in the South Island (DairyNZ.2012). The North Island had the highest number of herds at 76% with 30% concentrated in the Waikato region and 15% in Taranaki while the South Island accounted for 24% of national herds but with 37% of all cows in the country. The Waikato region had the highest dairy cow population at national level followed by North Canterbury. The distribution of dairy cows in New Zealand according to regions is shown in Figure 1.3.

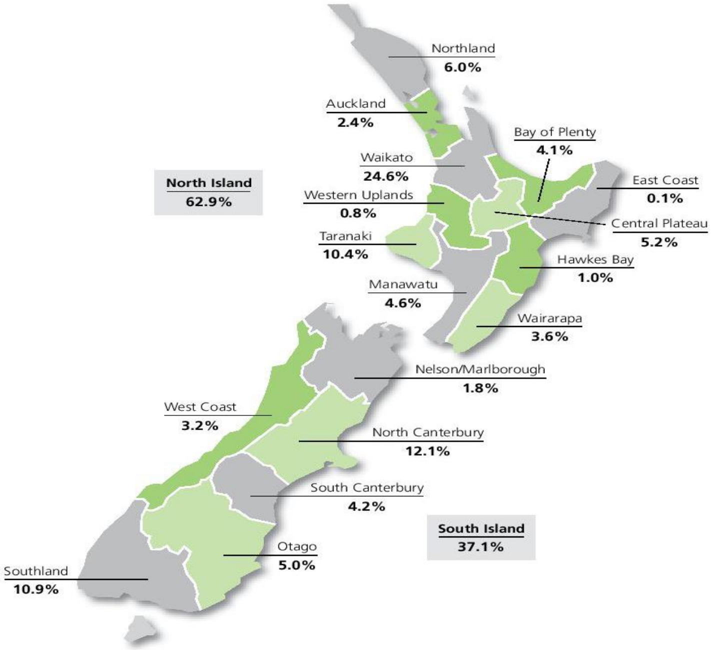

Figure 1.3 Regional distributions of dairy cows in the 2011/12 season. (Source - NZ Dairy Statistics 2011/12)

Friesian is the predominant dairy breed in New Zealand making up about 47% of the population, followed by 36% cross-breeds (mainly Friesian-Jersey crosses), 15% Jersey, 2% Ayrshire and a small proportion of other breeds (Stringleman and Scrimgeour.2012). It is important to consider several traits other than milk production when selecting for breeding and replacements. Selecting for milk production alone has resulted in offspring that are prone to increased metabolic demands and prolonged periods of negative energy balance (NEB) (Alawneh *et al.*2014). high producing cows eventually mobilize more body tissues during early lactation which could be seen as excessive live weight loss because of the negative correlation between yield and feed intake, meaning the feed intake is unable to cover the extra requirements for increased milk yield (Veerkamp *et al.*2000).

1.3 Energy balance in dairy cows.

Energy balance (EB) in dairy cows is defined as the difference between energy intake from feed and energy required for body maintenance, production and gestation (Alawneh *et al.*2012; Heuer.2000). NEB results in animals losing body condition. Metabolic and calving stress is thought to affect the fat-protein ratio (FPR) in milk. The FPR differs between cows and depends on the lactation stage. It is often high in early lactation when NEB is most likely to occur (Buttchereit *et al.*2010). The nutrient demand of lactation typically exceeds the dietary intake potential in the early postpartum period which often results in a varying extent of NEB (Patton *et al.*2006). High producing dairy cows mobilize their body fat, and to some extent, protein reserves in order to sustain their milk production which leads animals to enter a state of NEB until energy intake meets the output requirements (Knop and Cernescu.2009; Zurek *et al.*1995). Loss of energy in feed reduces the ability of rumen microbes to digest plant proteins and synthesise animal proteins, thus reducing the protein percentage in milk. The mobilisation of body fat increases non-esterified fatty acids (NEFA) in the liver and consequently the percentage of fat in milk. As the percentage of fat in milk increases and that of protein decreases, the FPR increases.

The resulting NEB and metabolic demands influence the postpartum interval to first ovulation and thereby affecting the interval to conception (Butler and Smith.1989) which eventually affect the reproductive potential of the affected cows. Conception rates are thought to be low in animals that experienced NEB prepartum and early postpartum because of the poor quality of oocytes generated during this stage (Knop and Cernescu.2009; de Feu *et al.*2009). Many studies have shown that while feed intake and milk production both increase after calving, maximum feed intake is only achieved some weeks after maximum milk yield (Garnsworthy.1988). A relationship that involves three components of dry matter intake (DMI), live weight and milk production comes into play throughout lactation. It is therefore biologically plausible that cows lose weight in early lactation as milk production increases rapidly and DMI cannot cope with the rising production demand. Figure 1.4 shows a relationship among the three components, and that live weight loss is the result of low dry matter intake and high milk production immediately after calving leading to NEB during the first 6-8 weeks of lactation (Heuer.2000). The energy status after calving is lowest at the time of peak milk production as shown in Figure 1.5. It would therefore be a challenging task to prevent NEB in cows that are naturally high producers because of the interplay of these factors.

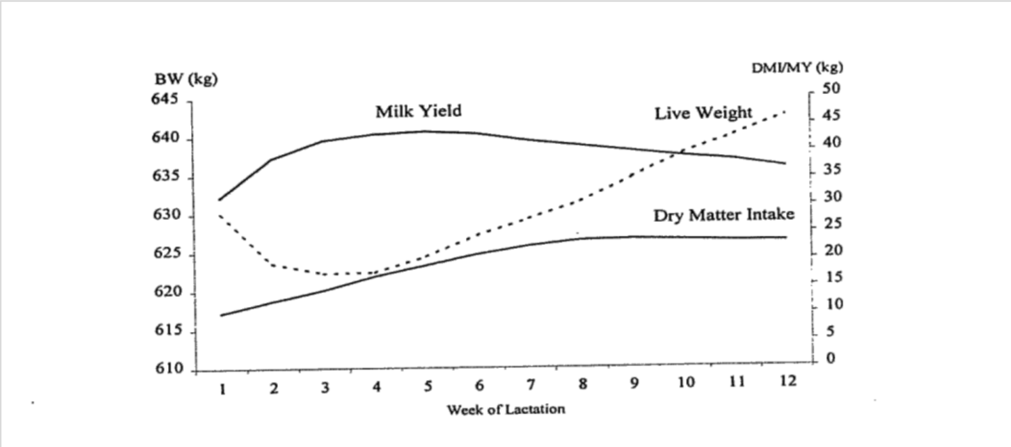

Figure 1.4 Dry matter intake (DMI), live weight (BW) and milk yield (MY) during the first 12 weeks of lactation.(Source: Heuer, C. 2000).

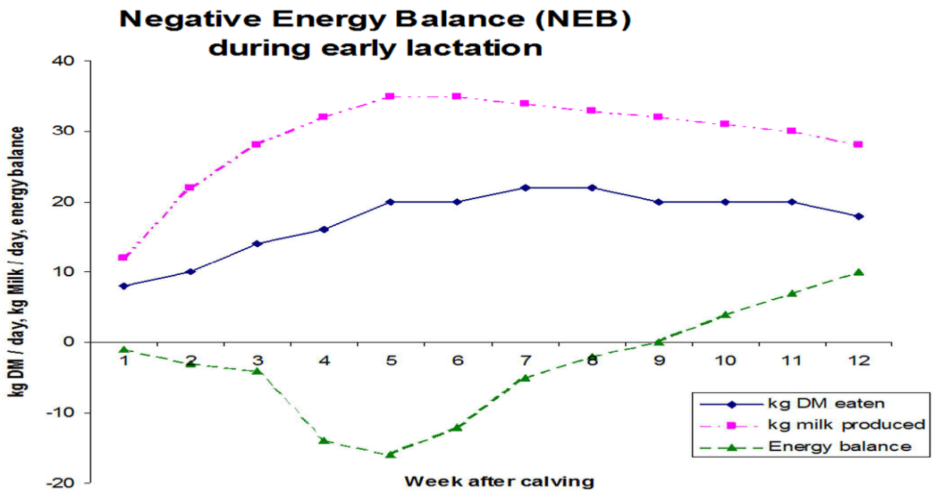

Figure 1.5 The relationship between milk yield, dry matter intake and energy balance in first 12 weeks of lactation.

Several adverse consequences of NEB have been investigated and documented which include metabolic disorders such as ketosis and acetonaemia, reproduction disorders such as anoestrous and infertility, and other health problems such as increased susceptibility to mastitis (Bareille and Noordhuizen.2008). Some of the methods proposed to diagnose NEB include estimating the difference between energy intake and energy requirements, body condition scoring and analysis of milk fat and milk protein especially in early lactation.

NEB is believed to have an influence on several production and physiological parameters in dairy cows as outlined in the causal diagram in Figure 1.5 and reviewed in the following sections. It would therefore be of paramount important to identify animals at risk of this condition and take mitigating action.

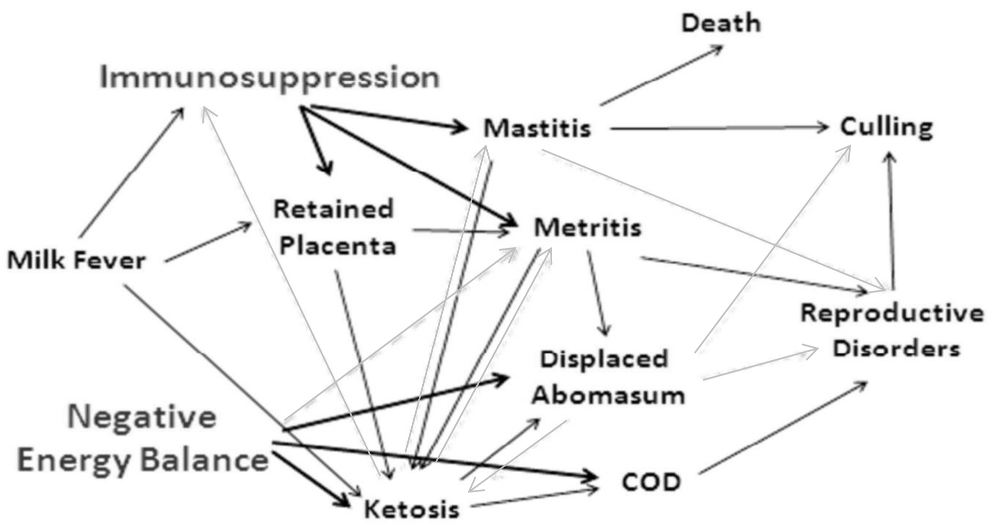

Figure 1.6 Complex interrelationships of negative energy balance – milk fever – immunosuppression on transition cow diseases (McKay B and Heuer 2000 *modified*). The light shaded lines represent weak associations among different parameters. COD - Cystic Ovarian Disease.

1.4 Relationship of NEB with Reproduction

Reproductive efficiency in cows plays a crucial role in ensuring profitability of the dairy enterprise because it determines the length of the inter-calving period and the productive lifetime. In the strictly seasonal calving system which is typical for over 90% of dairy farms in New Zealand, conception within planned or optimal times is a major determinant of culling, which contributes to financial losses. NEB during early lactation in dairy cows leads to alterations in metabolic state that has major effects on the production of insulin-like growth factor (IGF) and related metabolites (Fenwick *et al.*2008). Since IGF play an important role in follicular growth and embryonic development (Kirby *et al.*1996) it becomes evident that reproduction potential is affected in animals that enter a state of NEB. High producing dairy cows have been observed to be more prone to NEB shortly after parturition, a situation that can impair reproductive recovery because EB is negatively correlated with days to first ovulation after calving (Beam and Butler.1997). Therefore cows in NEB during early lactation would generally conceive later than expected.

Ovulation after parturition is usually first observed within three weeks in about 50% of healthy cows (Castro *et al.*2012) . Early resumption of ovarian activity should lead to high fertility which would increase the chance of cows achieving the 365 days reproduction cycle. The delays in onset of postpartum ovarian activity eventually limit the number of oestrous cycles before breeding, which could account for the decrease in fertility (Butler and Smith.1989). NEB therefore would be thought to act in a similar manner as under-nutrition in which ovarian activity is delayed which later affects secretion of follicular and luteinizing hormones (Zurek *et al.*1995). The magnitude and duration of the prepartum NEB status has a detrimental effect on reproduction and production performance in high producing dairy cows (Wathes *et al.*2007). The changes in biochemical, endocrinological and metabolic pathways are associated with delay of the first visible signs of oestrus, increased calving to first ovulation interval, decreased conception rates and prolonged calving interval (Rukkwamsuk *et al.*1999). An increased calving to first ovulation interval would definitely affect PSM to conception interval at the herd level since the PSM date would arrive with some animals not yet physiologically ready for servicing.

Body condition score decreases as body reserves are mobilized to compensate for NEB in early lactation leading to detrimental effects on the performance of the animal (Collard *et al.*2000). In as much as every dairy farmer would like to have high producing cows in his herd, care has to be taken in order to maintain a balance between production and reproduction performances. This is important to consider since cows would usually be kept in the herd until they are in parity five or sometimes higher. If then conception rates are way below expectations, culling and replacements increase, thus herd productivity in the subsequent season may get seriously affected. Some studies have shown that animals that suffered NEB failed to reach peak milk production in 16 weeks and eventually lost weight and had reduced conception rates (Rukkwamsuk.2010). Efforts and strategies to reduce negative effects of NEB should therefore be made before calving.

1.5 Relationship of NEB with cows' health status.

Further research has suggested that animals in NEB have a reduced immune response which later results in several negative events like mastitis, lameness, respiratory diseases and metritis (Rossi *et al.*2008; Moyes *et al.*2009). A study of effects of NEB on udder inflammation in Holstein dairy cows concluded that animals in severe NEB that had increased SCC in milk (van Straten *et al.*2009). SCC in milk, which can act as an indicator of subclinical mastitis, was observed to be higher in animals with four and more lactations (Syridion *et al.*2012). A study on expert opinion identified high SCC as one of the risk factors associated with preceding or subsequent subclinical mastitis in dairy animals (Lees and Lievaart.2013). Animals with FPR greater than 1.5 were at greater risk to be in NEB (Heuer *et al.*1999) especially in early lactation. It is therefore justifiable to assess the relationship between NEB and SCC because very low SCC on the other hand has been identified to be a strong risk for the development of clinical mastitis (Suriyasathaporn *et al.*2000) which becomes costly for the affected dairy enterprise due reduced production and cost of treatment.

Since animals in a state of NEB eventually lose weight, this could also contribute to the development of lameness. It has been demonstrated that animals with excessive weight loss postpartum had an increased risk of subsequent lameness (Alawneh *et al.*2014). Lameness is painful and will restrict the animals from normal movement and eventually feed intake, especially for pasture dependant feeding, which would lead to a chain of undesirable events including poor animal welfare. Left displaced abomasum (LDA) is a disorder that occurs mainly in high producing dairy cows postpartum (Esposito *et al.*), often without causing observable clinical signs. Studies have also shown a relationship between NEB and LDA postpartum (Cameron *et al.*1998). The causal pathway for LDA is not certain but thought to involve disorders of NEB, hepatic lipidosis and ketosis. Clinical ketosis leads to recumbency and is a consequence of an excessive mobilization of body fat by a cow. It is common in early lactation and characterised by hypoglycaemia and hyperketonemia (Esposito *et al.*) that later induce a decline in plasma insulin and mobilization of triacylglycerol deposits as NEFA.

Amounts of both NEFA and β-hydroxybutyrate (BH) in early lactation are believed to be normal (Daryl *et al.*2012). However, excessive amounts have been observed to increase the risk of disease, decreased reproductive efficiency and lower milk production. While being a strong contributor, NEB is only one of several possible causes for poor reproduction outcomes because other herd management factors are also involved, such as low DM availability through pasture prior to calving, insufficient maintenance of cow races, poor silage quality or other diseases. Both NEFA and BH at concentrations greater or equal to critical thresholds were seen to increase the risk of LDA by up to 10 times (Daryl *et al.*2012). Uterine diseases after calving have been seen in some studies to be higher in cows that experienced a greater degree of NEB postpartum and had decreased glycogen levels of intracellular polymorphonuclear neutrophils (Galvão *et al.*2010), which could be a major predisposing factor for disease.

Since NEB has effects on milk production, reproduction and immune system in dairy cows as outlined in the literature review above, dairy farmers should be on the look-out so as to reduce the impacts of these conditions especially in cows at high risk. The farmer should ensure availability of enough DM and provision of optimal and well balanced nutrition during the transition period as well as during early lactation (Geert.2013). This could be done by providing animals with energy dense rations in beginning of lactation to alleviate as much as possible fat reserve mobilization (Viturro and Altenhofer.2013). Efforts should be made to check in the stable to determine whether the calculated amounts are really being consumed by the cows and veterinarians should use their expert knowledge to determine the general health status of the animal and intervene when necessary (Geert.2013).

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2 Chapter 2: Analysis of NEB effects on Dairy Data from three regions in New Zealand.

2.1 Introduction

Negative energy balance (NEB) is of interest in the dairy industry because it does not only affect production and reproduction performances but is also an animal welfare matter. Animals in NEB usually loss body condition after calving usually occurring at 50 to 100 days post-calving (Roche *et al.*2009), which is a recognised animal welfare problem. There is appearing evidence that high yielding cows which loose body condition during periods of NEB become lame (Huxley.2013). Lameness is associated with animal welfare and has substantial negative effects on fertility performance and reproductive parameters, which would eventually lead to culling. Body condition score (BCS) may also be used as a valid indicator of animal welfare, but further research is required to determine effect of BSC change in cows (Roche *et al.*2009). Since most of New Zealand dairy products are for export markets, anticipated high standards of primary production, milk quality and animal welfare should be maintained.

The relationships between NEB and several other variables (reproduction, production and culling risk) in cows were assessed. An elevation of fat-protein ratio of 1.5 and above has been suggested to indicate a likely state of NEB (Heuer et al.1999) while other studies concluded that a decreased fat percentage during early lactation of first lactation heifers might serve as an indicator of energy balance (de Vries and Veerkamp.2000). The later however was based on a system of 11 herd tests per year whereas dairy herds in New Zealand are only being tested up to four times per milking season. A period of NEB is a common occurrence affecting at least 80% of dairy cows. NEB is as a result of insufficient energy intake (usually caused by a depression of appetite) relative to requirements resulting in lipolysis, and to some extent, proteolysis to compensate for the energy deficit (Sovani *et al.*2000; Rukkwamsuk *et al.*1999). High-producing dairy cows that are over-conditioned at calving have been observed to get into a more severe NEB postpartum compared to animals with normal body condition. This condition could eventually induce changes in biochemical, endocrinological and metabolic pathways (Rukkwamsuk *et al.*1999). This could then lead to poor performance of dairy cows in the postpartum period since these pathways are responsible for production, reproduction and health maintenance. Body condition scores (BCS) reduce as reserves are mobilized to compensate for NEB which has been associated with reduced conception rates, especially for animals that got affected in their first month of lactation (Collard *et al.*2000). In New Zealand however, more cows tend to be under-conditioned than over-conditioned at calving (Anonymous.2014). No information exists to date about the impact of under-conditioning on the production and reproduction performance, on the risk of metabolic or production diseases, on culling, or on compromising animal welfare in seasonally calving dairy cows in New Zealand.

The adverse effects of NEB can disrupt management targets like a compact seasonal calving period. A compact calving period is achieved by an intensive 8-12 weeks breeding season program in which a calendar date is predetermined as the "planned start of mating" (PSM) followed by a "Planned Start of Calving" (Verkerk.2003). Other factors have been associated with PSM to conception interval, which include parity, milk protein percentage, breed, clinical mastitis and season (Alawneh *et al.*2012). When reproductive performance is significantly reduced, these targets can often not be achieved leading to loss of business and unfavourable decisions of early culling due to infertility or production diseases such as ketosis, mastitis or lameness. It would therefore be prudent to prepare dry cows for high feed intake in early lactation by winter feed planning to achieve optimal body condition at calving, adequate water supply, and a reserve of high-quality silage so as to prevent NEB (Heuer *et al.*2001).

Figure 2.1 shows the hypothesised pathways of NEB with various physiological and production aspects of the dairy animal. The causal diagram shows that NEB predisposes animals to subclinical mastitis (high SCC) which could affect milk production and fertility levels in turn increasing the risk of involuntary culling to affected cows. High SCC could be an indicator of intrammammary infection and could occur at different stages of lactation (DeVries *et al.*2012; Heuer *et al.*2001; Vissio *et al.*2014). Animals in NEB are generally believed to be high milk producers which could be a source of stress. When the dairy animal is not in good health, pregnant rates at herds are affected leading to increased risk of culling.

This study was aimed at evaluating the effects of NEB in affected animals and relationships in the hypothesised pathways shown in 2.1 were explored. Multivariable models were used assess the effect of each covariate, with NEB being the primary variable of interest.

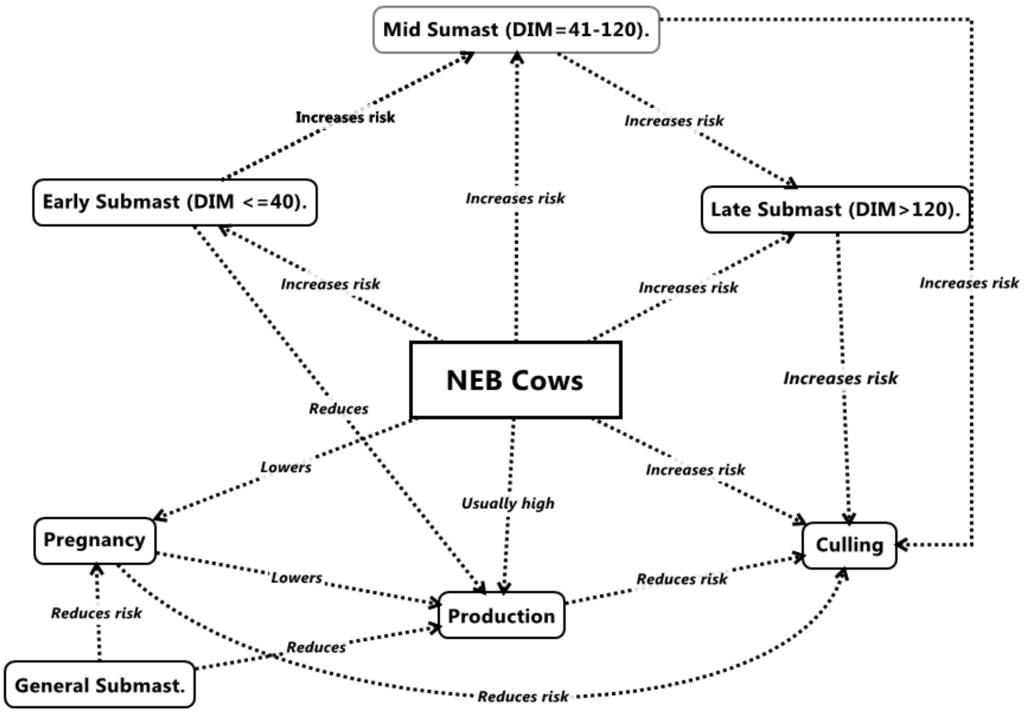

Figure 2.1 The hypothesised causal diagram of NEB in affected dairy cows. Submast=subclinical mastitis (elevated SCC of 250,000 and above), DIM=days in milk, NEB=negative energy balance.

2.2 Material and Methods

2.2.1 Herds and Animals.

Data used in this study originate from a recent study conducted in 2002 to evaluate the predictive value of BVDV antibodies in bulk tank milk for 724 dairy herds; data collection methods were described there (Thobokwe et al., 2004). Briefly, a simple random sample of 800 herds was targeted from a population of dairy herds in the North Island of New Zealand in a study estimating the prevalence of herds infected with bovine viral diarrhoea virus (BVDV). A total of 724 herds were randomly sampled for BVDV bulk tank milk (BTM) antibody testing in March 2002. The results were compared to the antibody status of 15 randomly selected calves and cows from a sub-sample of 50 herds tested for BVDV-antibody indicating the presence of one or more persistently infected calves and therefore the herd infection status. The study resulted in a cut-off point of 80%INH BVDV-antibody in BTM indicating a likely presence of one or more persistently infected high shedding animals.

This chapter presents an analysis of data for the 2001/2 season from herd tests by LIC in three regions of the North Island of New Zealand, namely Bay of Plenty, Northland and Waikato. It involved three dairy breeds - Friesian, Jersey and cross-breeds. The data included information about cows' reproduction, production and culling. Reproduction data included mating, calving, number of services and pregnancy test information. Production data included milk volume produced in the morning and evening of each test day, the percentage of milk solids (fat and protein fat) and the count of somatic cells per millilitre of milk per day. Culling information was about the fate of the animals during the observation period which run from 2001 to 31st December 2004. Details relating to removal from the herd were entered against each animal as to whether it got culled, sold or died within this period and reasons given for each fate. Other variables were derived from the data that was initially collected. These included fat-protein corrected milk (FPCM), fat-protein ratio (FPR), days in milk (DIM) at each herd test, first service conception, planned start of mating (PSM) to conception interval and subclinical mastitis.

2.2.2 Data validation

Available herd information from 632 herds (267,171 cows) was downloaded from the Livestock Improvement Centre (LIC) database and validated using MS-ACCESS. A total of 2,316 cows from 3 herds were initially excluded from the database for the following reasons: 84 cows had duplicate entries (same lifetime identification and same herd code) but were listed with a different exit reason; a further 1,640 cows were allocated to two different herds with two different results from the bulk tank milk testing and 592 animals were allocated to two different herds at the same time with different BTM test results, thus were regarded as entry errors. Other categories of animals removed from the study included 126 animals removed due the breed information missing, 24 animals with missing age information, 16 animals due to milk production records missing and 767 animals with SCC recorded as zero, which was considered as error in data entry. A total of 185, 050 from 590 herds had calving dates from 01 June 2001 to 31 March 2003, and 146, 038 animals from 587 herds had mating dates from 01 September 2001 to 31 May 2002.

This study targeted cows that had complete milk production Information and reproductive performance for the 2001 to 2002 lactation and breeding season as available from the LIC database. A total of 132,270 animals from 543 herds had complete milk production records. Twenty two cows were excluded because they had abnormally high FPCM of 60kg and above and 2,288 cows from 3 herds were excluded because the milk record per milking session was less than 3 litres which was considered to be abnormally low. Five herds consisting of 13 cows were removed because they had less than six animals each which were treated as outliers. Finally, a total of 129,947 animals from 535 herds remained in the data set for this study. Breeds that were less than 80% pure were classified as cross-breeds. The cross-breed category had a small proportion of the Ayrshire breed. The distribution of animals across the regions and the breed types, together with the breed percentage in each particular region are shown in table 2.1 below. The Friesian was the dominant breed in all the three regions followed by the cross-breed and lastly the Jersey breed.

Table 2.1 Distribution of cows according to breed type and region of farm location.

	Friesian	Jersey	Cross-breed	Totals	% per Region
Bay of Plenty	21,448	3,792	5,041	30,281	23%
(Breed % in the Region)	*(71%)*	*(13%)*	*(17%)*		
Northland	11,348	3,567	5,792	20,707	16%
(Breed % in the Region)	*(55%)*	*(17%)*	*(28%)*		
Waikato	43,197	17,122	18,640	78,959	61%
(Breed % in the Region)	*(55%)*	*(22%)*	*(24%)*		
Totals	**75,993**	**24,481**	**29,473**	**129,947**	

2.2.3 Variables of interest

The variables of interested in the study included adjusted production of milk solids or FPCM, FPR, SCC, age (parity) of the animals, DIM at time of herd test and pregnancy rates. Other variables included PSM to conception interval for 2001 breeding season, first-service-conception rate for 2001 breeding season, calving order of each animal, breed type, region of farm location, culling rate for the 2002 season and overall culling rate for period ending 31/12/2004.

Animals were grouped into six parity categories depending on their age. The minimum parity category consisted of animals that were less than three years as parity one. A one year increase in age corresponded to a unit increase in parity. Parity six consisted of animals that were eight years or older, hence in their 6th or higher lactation. The numbers of animals at each herd test by parity category are shown in Table 2.2, with parity 3 being the median category. The average herd size for all the 535 herds was 422 with the minimum of 16 and maximum of 1,573 lactating animals.

Table 2.2 Parity categories of animals that were tested at each herd test.

	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5	Parity 6	**Total Tested**
Test 1	23648	20913	18400	16244	14225	36517	**129947**
Test 2	21609	19380	17223	15128	13247	33699	**120286**
Test 3	18126	16575	15116	13343	11494	28447	**103101**
Test 4	9516	9813	9968	9170	7708	17816	**63991**
Totals	**72899**	**66681**	**60707**	**53885**	**46674**	**116479**	**417325**

2.2.4 Fat-protein corrected milk.

Two milk production records of the morning and afternoon were recorded on the test day and the percentages of fat and protein measured. The two volumes were added to give the total milk volume production per day for each cow and averages calculated for percentage fat and protein per day, respectively. The FCPM for each cow was corrected at 4.5% fat and 3.2% protein and was calculated as:

$$\boldsymbol{FPCM = milk\ volume * fat\ percentage/4.5 * protein\ percentage/3.2};$$

FPR was calculated by dividing the milk protein percentage into the milk fat percentage. A FPR of 1.5 and above for first herd test was set as an indicator of animals in NEB as has been suggested by other studies (Heuer et al.1999). In this study only animals with a FPR of 1.5 and above during their first milk test were considered to be in NEB regardless of the ratios on subsequent tests and a binary variable (yes/no) for NEB was developed. Testing times ranged for one to four tests with an average of two tests per animals. FPCM was compared across many variables using boxplots and scatterplots to appreciate the production trends.

SCC for each individual cow was measured from quarter milk pooled as one composite sample on the day of testing. SCC was then used as a proxy indicator of intra-mammary infection or subclinical mastitis (DeVries et al.2012). An elevation in SCC in milk of greater or equal to 250,000 cells/ml was used as the cut-off point for subclinical mastitis, which was transformed to a binary variable (yes/no).

The DIM when the individual milk tests were done were divided into three categories as follows; 0 to 40, 41 to 120, and over 121 as stages 1, 2 and 3, respectively. For the first milk test, a total of 54,549 animals were tested in the DIM stage 1, 70,709 in the stage 2 and 4,702 animals in the stage 3. The rest of the information for other tests is summarised in Table 2.3. DIM category was included in models to adjust for the confounding effect of lactation stage on the association between FPR and outcomes like milk production and SCC

Table 2.3 Number of cows tested at different test numbers and DIM summaries for different DIM stages.

		Test 1	**Test 2**	**Test 3**	**Test 4**
DIM stage 1 (0-40 days)		54,540	117	3	-
	Average	*24.9*	*36*	*18*	
	Median	*26*	*37*	*12*	
	Minimum	*1*	*8*	*10*	
	Maximum	*40*	*40*	*33*	
DIM stage 2 (41-120)		70,707	90,688	2,360	465
	Average	*59*	*94*	*104*	*106*
	Median	*56*	*96*	*110*	*107*
	Minimum	*41*	*41*	*57*	*85*
	Maximum	*120*	*120*	120	120
DIM stage 3 (above 120)		4,700	29,481	100,738	63,526
	Average	*187*	*151*	*171*	*221*
	Median	*190*	*135*	*171*	*222*
	Minimum	*121*	*121*	*121*	*121*
	Maximum	*289*	*304*	*325*	*320*

Histograms showing the distribution of DIM stages for first milk tests and DIM for all the four tests are shown in Figures 2.2 and 2.3, respectively. The test times showed overlaps across the milk test times.

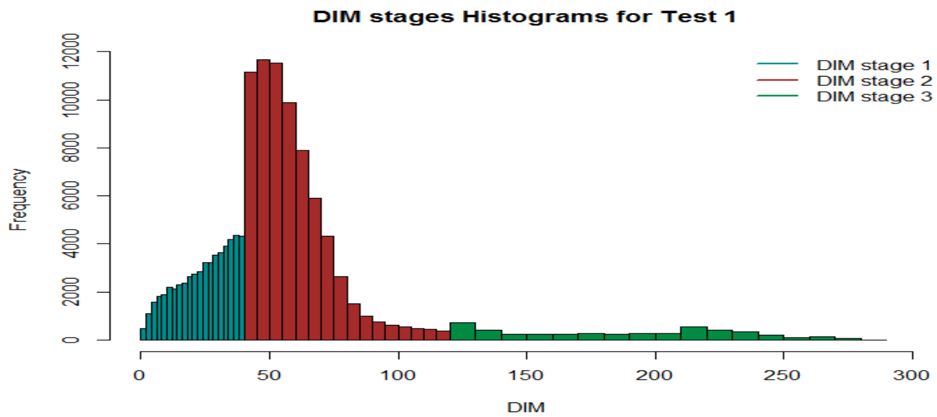

Figure 2.2 Histogram of DIM stages for the first milk test on scale of 300 DIM.

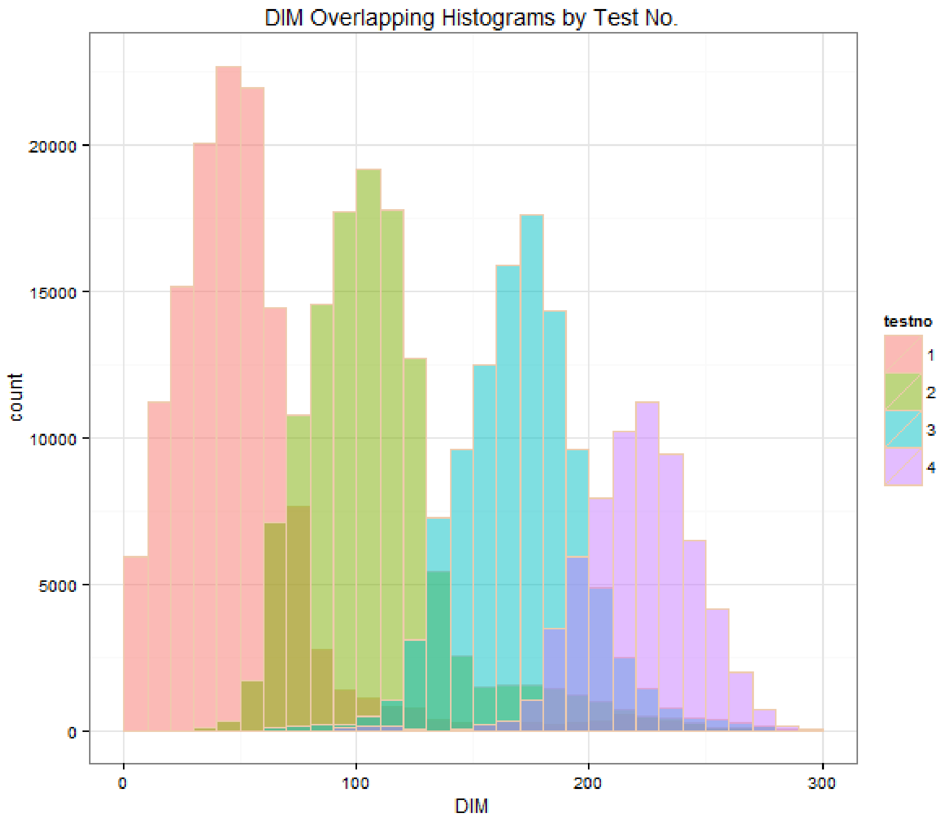

Figure 2.3 Overlapping histograms for the four milk tests on a DIM scale of 0-300.

Cows were categorised in three calving-orders depending on the time they calved during the calving period. The calving range was obtained by subtracting the first calving date from the last calving date in the herd and then divided by three to obtain calving-order. The first third of the range or calving period was classified as first order and so on. The distribution of animals in each calving order at each herd test is shown in table.

Table 2.4 Animals according to calving order at each milk test.

	Test 1	Test 2	Test 3	Test 4
Calve order 1 (1st third)	43,539	40,168	34,192	20,942
Calve order 2 (2nd third)	43,374	40,287	34,658	21,689
Calve order 3 (3rd third)	43,034	39,831	34,251	21,360

2.2.5 Pregnancy rate, planned start of mating to conception interval and culling rates.

The calculation of the reproductive performance indicators required cows with complete records of calving and mating dates in 2001/02 as well as calving or removal dates of the subsequent season 2002/03 which was done for all 129,947 animals and 535 herds. The gestation period was calculated as the difference between the last mating date in the 2001/02 season and the calving date in the subsequent season. A key was derived which defined the pregnancy status (Yes/No), the first service conception (Yes/No) and the time between calving and conception for each animal. Where no information about the pregnancy outcome was stated in the LIC database, a gestation period of 270-300 days was considered as *normal,* a gestation period of 250-270 days as *Induced* and a gestation period of less than 250 days as *Abortion (Macmillan and Curnow.1976)*. Cows with those gestation periods were defined as having conceived on the mating date if they only had one mating in that season. A first-service-conception was defined for animals calving, aborting or being induced in 2002/03 season with only one preceding service or mating date.

The PSM was taken as the first date of mating in each respective herd, but this was set as 10/10/2001 for herds where mating dates were not recorded for 2001. A period interval of PSM to conception for each animal was calculated by obtaining the difference between the PSM date and conception date for the 2001/2 breeding season. Animals with no information about calving dates of the subsequent season were considered as not pregnant, if they were culled or sold due to infertility. The animals without subsequent calving dates that remained until the end of the study period (31/12/2004) were consider to be pregnant, suggesting they were pregnant and therefore not removed. If animals calved in 2001 and had their subsequent calving date in 2003, they were excluded from the analysis of the PSM to conception interval for the 2001/2 season.

The calving-conception interval was calculated as the difference between the calving date and the subsequent last mating date in the season 2001/02. For animals which had a gestation period longer than 300 days and no stated pregnancy outcome in the LIC database, we assumed another mating date in between, which was calculated and then used for the calving-conception interval calculation. The maximum culling time at risk for the year 2002 was set as 31st December 2002.

2.2.6 Descriptive analysis.

The descriptive statistics were calculated for binary and continuous variables (obtaining measures such as means, standard deviation, minimum and maximum measures). Two by two tables were constructed for the binary variables with negative energy balance taken as the exposure variable. Outcome variables included subclinical mastitis, first service conception, pregnancy, risk of culling in the 2002 breeding season and overall culling risk. Crude measures of association analyses were done at bivariate level to determine association between the exposure and the outcome variables using the R software and the epiR package (Mark Stevenson *et al.*2013). Measures of association were also assessed between subclinical mastitis and pregnancy, taking the former as the exposure variable.

2.2.7 Statistical Analysis

A total of six multivariable mixed models were developed using the R software (RCore Team.2013). Two of the models were linear mixed effects which had continuous outcome variables, FPCM and the SCC transformed to the log scale which were done using nlme package (Pinheiro *et al.* 2013). The lme4 package (Bates *et al.* 2013) was used for three generalised mixed logistic regression models which had binary outcome variables that included culling risk for 2002 season, pregnancy and subclinical mastitis. The total variances for linear mixed models were obtained by adding the random and the fixed effects variance components. The latent variable approach of adding the constant variance π2/3 (3.290) to the random effects variance was used to get the total variance for mixed logistic models (Dohoo *et al.*2009). The intra-cluster correlation (ICC) which measures the similarities within a herd was obtained by dividing the total variance into the herd random effect variance. The data used in all the models were for test one only.

Survival analysis using the cox proportional hazard model with a gamma-frailty term was performed for PSM to conception interval to assess effects of various variables on the time to event. The hazard in this model was animals conceiving during the 2001/02 breeding season. The observation period for conception ended on 31st May 2002, which was estimated as the end of the 2001/2002 breeding season. The Kaplan-Meier curves (Kaplan and Meier.1958) were used to assess the effects of NEB, subclinical mastitis, breed and region on PSM to conception interval. The log rank test was used to quantitatively compare survival probabilities among the four mentioned strata within each covariate.

The Wald and partial likelihood ratio tests were used to assess the significance of the variables in all the models with the critical value set at $P \leq 0.05$. The relationships between predictors and outcome variables were assessed for each specific model with NEB being the primary variable of interest in all the models. Two way biologically plausible interactions were assessed and only included in the final model when they were found to be significant.

Several variables of interest were either obtained or developed from the dataset which are shown in Table 2.4 with their abbreviations. The generic model for all the models was:

$$Y = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \cdots + \beta_k x_{ki} + \mu_j.$$

Where y = fat-protein corrected milk, or log scale of SCC, or logit-transformed for pregnancy variable, or logit-transformed subclinical mastitis variable, logit-transformed culling for 2002 or PSM to conception interval. The X= coefficients of various variables were those shown in table 2.4 and μ was the random effect (herd identity).

Table 2.5 Variables of interest included in the models, their class and the abbreviations used during model building.

Variable	Class	Abbreviation in model equations.
Negative energy balance.	Binary	NEB
Subclinical mastitis.	Binary	Submast
Days in milk stage (1, 2 and 3).	Categorical	dim_stg
Fat-protein corrected milk.	Continuous	FPCM
Herd identity.	Categorical	Herdid
Herd size	Continuous	Herdsize
Calving order (1 to 3) – period when cow calved.	Categorical	Calvorder
Planned start of mating to conception interval	Continuous	PSM_concep
Somatic cell count – transformed to log scale	Continuous	logSCC
Parity – 1 to 6	Categorical	Parity
Pregnancy	Binary	Pregnancy
First service conception	Binary	Firstcon
Number of services per conception	Continuous	Services
Culled in 2002	categorical	Culled02
Breed – Friesian, Jersey and Mixed	Categorical	Breed
Region – Bay of Plenty, Northland and Waikato	Categorical	Region

2.3 Results

2.3.1 Descriptive statistics

Data from a total of 129,947 animals from 535 herds in three regions were available and subjected to data analysis. The number and proportion of cows for NEB, subclinical mastitis, pregnancy, first service conception, and culling and the crude association with NEB are shown in table 2.5.

Table 2.6 Numbers and percentages of cows in described state over the total population for each variable and their crude association with NEB, and association of subclinical mastitis and prenancy. Subclinical mastitis and NEB were for first herd test only.

Variable	Number of cows	Cows in the state	Percentage	Association with NEB		
				CRR	95% CI	P value (*Chi square*)
Negative energy cows	129,947	12,118	9.3%			
Sub clinical mastitis cows	129,947	16,291	12.5%	0.9`9	0.94, 1.04	0.63
Pregnant cows	129,947	115,009	88.5%	1.01	1.00, 1.02	0.00145
First service conception	129,947	67,220	51.7%	0.99	0.97, 1.01	0.31
Culled animals in 2002	129,947	26,540	20.4%	1.02	0.98, 1.06	0.38
Culled animals overall	129,947	55,495	42.7%	1.03	1.01, 1.06	0.0017
Subclinical mastitis association with pregnancy				0.97	0.96, 0.97	<0.0001

CRR- crude relative risk, CI – Confidence interval

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A total of 9.3% of animals were classified to be in NEB and 12.5% as having had subclinical mastitis. Pregnancy rate was at 88.5% while 51.7% conceived at first service. Measures of association at crude level showed that there was no association between NEB and the occurrence of subclinical mastitis with a relative risk (RR) of 0.99 (P = 0.53). Therefore, overall animals in NEB did not have a higher chance of developing subclinical mastitis than animals without NEB. Animals in NEB were more likely to get pregnant with a relative risk of 1.01 (P = 0.0014). First service conception and culling risk for 2002 were not associated with NEB. There was however a significant association between NEB and the overall culling at the end of the study period which resulted in crude RR of 1.03 (CI: 1.03 to 1.06).

A total of 26,540 cows were removed from herds in 2002 representing 20.4% of the total study population in which animals died, or were culled or sold. Animals were culled due to various reasons among which the prominent included non-pregnancy at the end of 2001/2 breeding season, low production, old age, high SCC, clinical mastitis, lameness and other causes that were not specified in the data set. A total of 10,175 animals representing 38.3% of culled animals were removed but without reasons given. The summary of numbers of animals removed from herds and reasons for removal with the corresponding percentages for the remaining 61.7% of culled cows are outlined in table 2.6. The reasons for removal were calculated on a 100% basis to show the burden of each in the herds

Table 2.7 Summary of cows removed from herds in 2002 and primary reason for removal with percentage contribution for each.

REASON FOR REMOVAL	CULLED	DIED	SOLD	Grand Total	Total Percentage
Non Pregnant	6,116	6	1,118	7,240	44.2%
Other miscellaneous reasons	2087	640	457	3,184	19.5%
Low production	1,277	436	651	2,364	14.4%
Old age	1,047	14	107	1,168	7.1%
High cell count	466	6	54	526	3.2%
Mastitis	455	24	36	515	3.1%
Feet or leg problems	355	29	11	395	2.4%
Culled/died injury or accident	199	1	14	214	1.3%
Late calving	194		56	250	1.5%
Udder breakdown	168	3	21	192	1.2%
Abortion	150		11	161	1.0%
Unsuitable udder/teats	142	2	12	156	1.0%
Totals	12,656	1,161	2,548	16,365	

For cows with accompanying reasons for removal, it could be seen that non-pregnancy at the end the breeding season had the highest burden which was at 44.24% followed by low production at 14.4%. A total of 3,184 animals were removed due to several miscellaneous reasons representing 19.5% of those with given removal reasons. Old age contributed 7.1% to culling, high SCC 3.2% and clinical mastitis 3.1%. Some of the causes of culling included lameness (2.4%), injury or accident (1.3%), late calving (1.5%), udder breakdown (1.3%), abortion (1.17%) and unsuitable udder (1.2%). Some of the causes included in miscellaneous category contributed less than 1% of the culled animals, such as unstable temperament, bloat, slow milking cows, dystocia cases, milk fever, cancer, blind quarter, Johnes' disease and spring eczema.

The six highest causes of removal from herds were almost consistent and comparable in terms of importance across all the three regions (Figure 2.4) and breed (Figure 2.5).

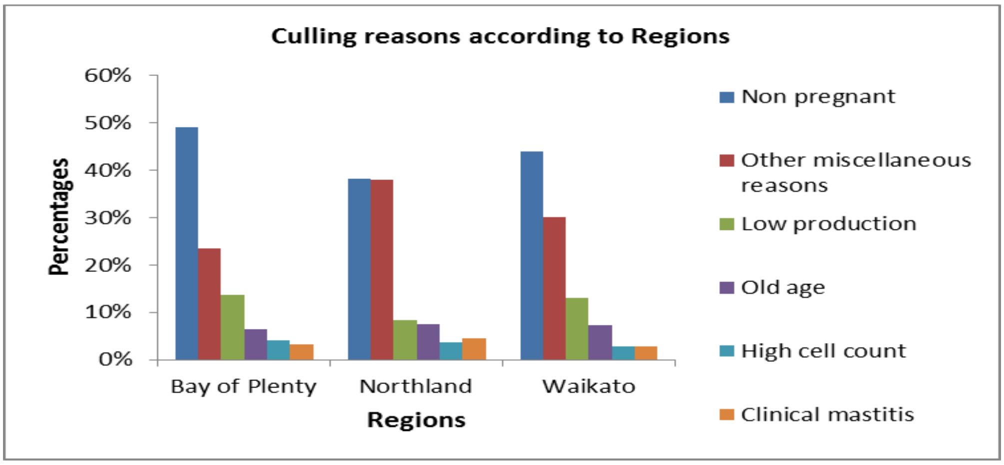

Figure 2.4 Percentages of common causes of culling according to regions.

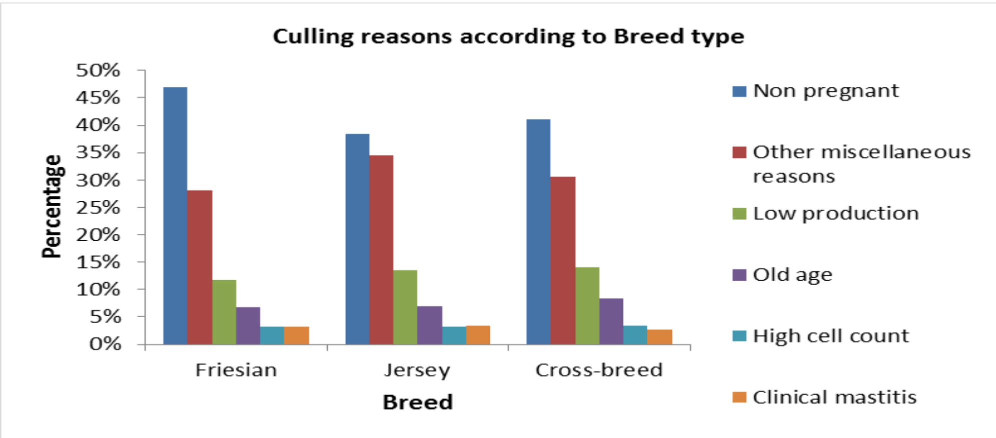

Figure 2.5 Percentages of common causes of culling according to animals breed.

The means, standard deviations and the ranges for the continuous variables were calculated as shown in Table 2.8 for the first milk test and for the tests.

Table 2.8 Summaries of means, standard deviations and range (minimum min, maximum max) of study variables for herd test one (129,947 cows) and overall observations (417,325 observations).

	Mean	SD	Min	Max
Milk production overall	20.4	6.3	4.1	59.8
Milk production Test 1	24.1	6.7	4.3	59.8
Milk production test 1 NEB+	26.4	7.7	5.6	59.4
Milk production test 1NEB-	23.8	6.3	4.3	59.8
Milk fat (%) overall	4.76	0.91	1.04	11.64
Milk fat (%)test 1	4.48	0.80	1.04	11.26
Milk fat(%) test 1 NEB+	5.75	0.73	2.66	11.26
Milk fat(%) test 1 NEB-	4.36	0.69	1.04	8.45
Milk Protein overall	3.63	0.40	1.34	9.99
Milk Protein test 1	3.56	0.36	1.34	9.9
Milk Protein test 1 NEB+	3.56	0.37	1.34	5.44
Milk Protein test 1 NEB-	3.56	0.36	1.71	9.99
Fat-protein ratio test overall	1.31	0.18	0.11	3.04
Fat-protein ratio test 1	1.26	0.19	0.11	3.04
Fat-protein ratio ≥ 1.5 test 1	1.62	0.14	1.50	3.04
Fat-protein ratio≤ 1.5 test 1	1.23	0.15	0.11	1.49
Somatic cell count ('000) overall	197	517	1	9999
Somatic cell count ('000) test 1	185	586	2	9999
*Somatic cell count ('000) T*1 NEB+	190	628	2	9999
*Somatic cell count ('000) T*1 NEB-	185	582	2	9999
PSM to conception	27	22	0	180
Days in milk overall	122	70	1	300
Days in milk for test 1	49	35	1	148
Herd size at testing	422	261	1	1573

PSM - Planned start of mating, NEB – Negative Energy Balance.

The means of milk production at herd level shows that the animals in NEB at first test had a higher mean production (26.5kg) than animals that were not (23.8 kg). Similarly the mean production at first test (24.1 kg) was higher than that for all milk tests combined (20.4kg). Animals that were not in NEB at first test showed a higher mean production compared to the overall mean for all the tests. This showed that high producers were more likely to suffer from NEB compared to the lower producers. The standard deviation for the higher producers (NEB+) was also higher than the lower producers (NEB-) even though the minimum and the maximum production were comparable.

The mean fat percentage in milk for animals in NEB (5.75%) was higher than those that were not (4.36%), even though the mean protein percentage (3.56%) was the same for the two groups. The mean fat-protein ratio at first test was however lower than the mean ratio for all the tests. The main impact on the differences in FPR was the fat percentage component because the fat percentage differed significantly between the two categories of NEB whereas the protein percentage did not.

Table 2.9 shows a comparison of the means of the variables across all the four herd milk tests. The means of milk fat, milk protein, FPR, SCC and subclinical mastitis were generally increasing with the subsequent milk herd tests. FPCM on the other hand was decreasing in relation to subsequent herd tests, which equally followed an increase in days in milk. This summary could be used to visualise variable trends with increasing days in milk, which would suggest that somatic cell count is generally high towards the end of lactation. The number of observations from which these statistics were drawn from varied across the different milk tests with test one having 129,947 observations while test four had 63,991 observations.

Table 2.9 Summaries of means for all the four milk herd tests.

	Overall	Test 1	Test 2	Test 3	Test 4
Observations	417,325	129,947	120,286	103,101	63,991
FPCM	20.4	24.1	20.2	18.1	16.9
Milk Fat	4.76	4.48	4.65	4.88	5.27
Milk Protein	3.62	3.56	3.54	3.64	3.86
Fat-Protein Ratio	1.31	1.26	1.31	1.34	1.36
NEB					
SCC	197	186	183	213	224
Subclinical mastitis	15.9%	12.5%	13.6%	18.3%	23.1%
Days in milk	122	49	108	170	220

Comment [c2]: Variable of interest

The mean planned start of mating to conception was 27 days and third quartile being at 39 days and maximum at 180. A breakdown of conception rates in two weeks intervals after PSM stratified by region and overall conception is shown in Table 2.10, and in Figures 2.7 and 2.8. The overall six weeks in-calf rate was 68.8% and the overall conception rate was 88.6% with small variations across regions. The PSM was comparable at two week intervals across regions, parity and breed type. The planned start of mating date and early breeding are very important to enforce in dairy industry because this could help cows attain the required/targeted 365 days breeding cycle in a year. PSM and a limited mating period are tools to ensure that cows calve close together in spring due to the availability of grass after winter rains when cows did not require much energy during the dry period.

Table 2.10 Cumulative proportion of cows that conceived at two week intervals after PSM.

Two Week Interval	Bay of Plenty Region	Northland Region	Waikato Region	Overall Conception rates
2	33.4%	30.7%	32.9%	32.3%
4	56.4%	52.9%	55.6%	55.0%
6	70.3%	66.8%	69.2%	68.8%
8	79.7%	77.4%	79.1%	78.7%

10	89.0%	88.0%	89.0%	88.6%

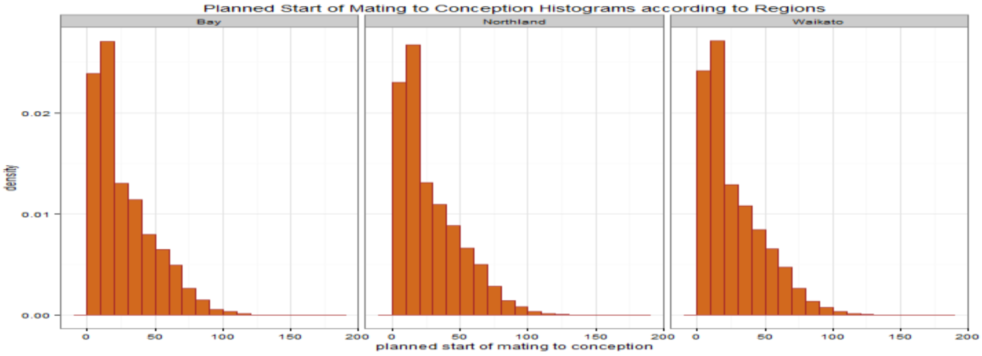

Figure 2.6 Density histograms of the PSM to conception interval according to regions.

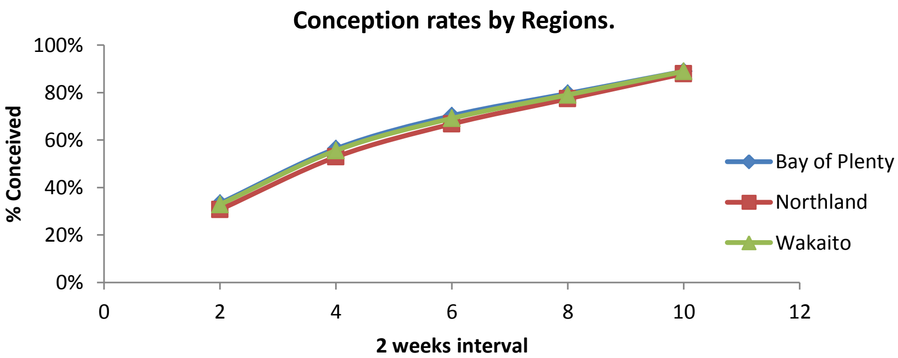

Figure 2.7 Percentages of cows that conceived at two week intervals in comparison to the planned start of mating to conception clustered according to region type.

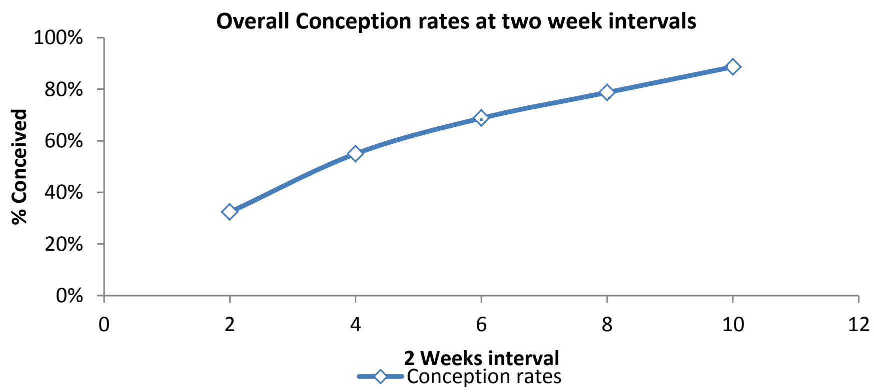

Figure 2.8 Percentages of animals that conceived at two week interval in comparison to the planned start of mating to conception.

The mean of DIM at first test was 49 days with a standard deviation of 35 days and maximum of 148 days. The mean herd size for first herd test was 422 with a standard deviation of 261 with minimum and maximum of 16 and 1573 cows, respectively.

All boxplots for FPCM versus test number (Figure 2.9), breed (Figure 2.10), region (Figure 2.11) and subclinical mastitis (Figure 2.12) indicated a constant increase in milk production with increase in parity number from parities one to five except a decrease in the last parity category of older animals (parity 6+). Animals in a NEB seemed had a consistently higher production across all categories of test number, region, breed and parity. Milk production was highest during the first milk test and was decreasing with subsequent tests. The production was highest at parities three, four and five across all the four strata of test number, breed and region. The Bay of Plenty and the Waikato regions had comparable production levels whereas Northland had a lower overall production compared to the other two regions.

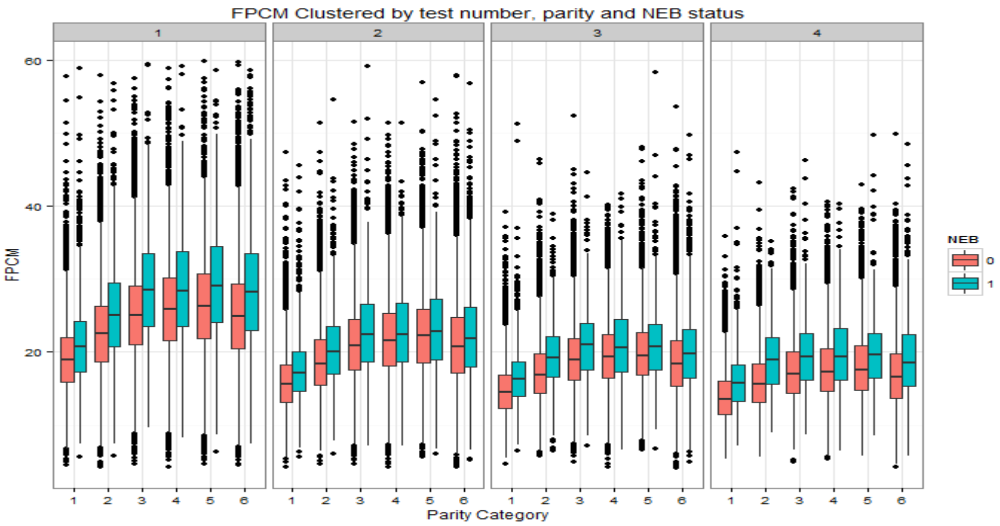

Figure 2.9 Boxplots of FPCM clustered by herd test number, Parity and NEB. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values

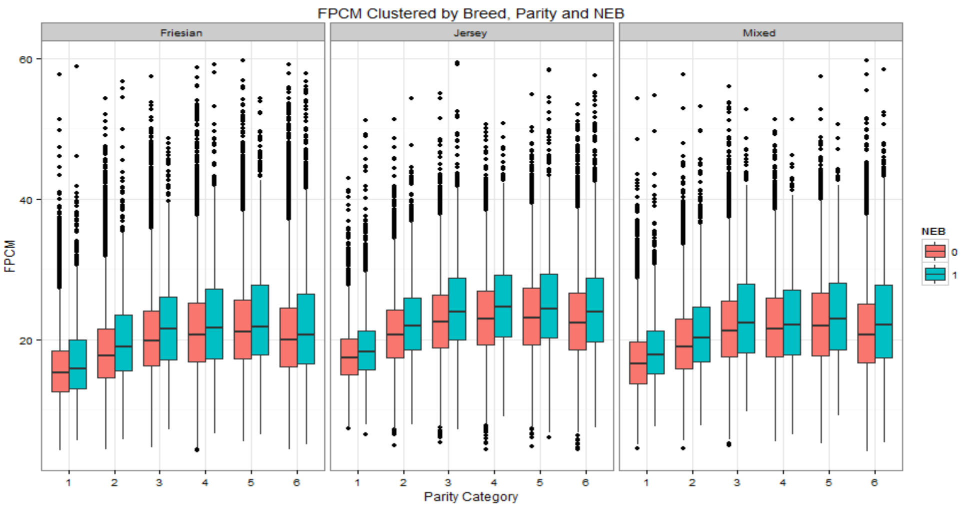

Figure 2.10 Boxplots of FPCM clustered by Breed, Parity and NEB. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extrem

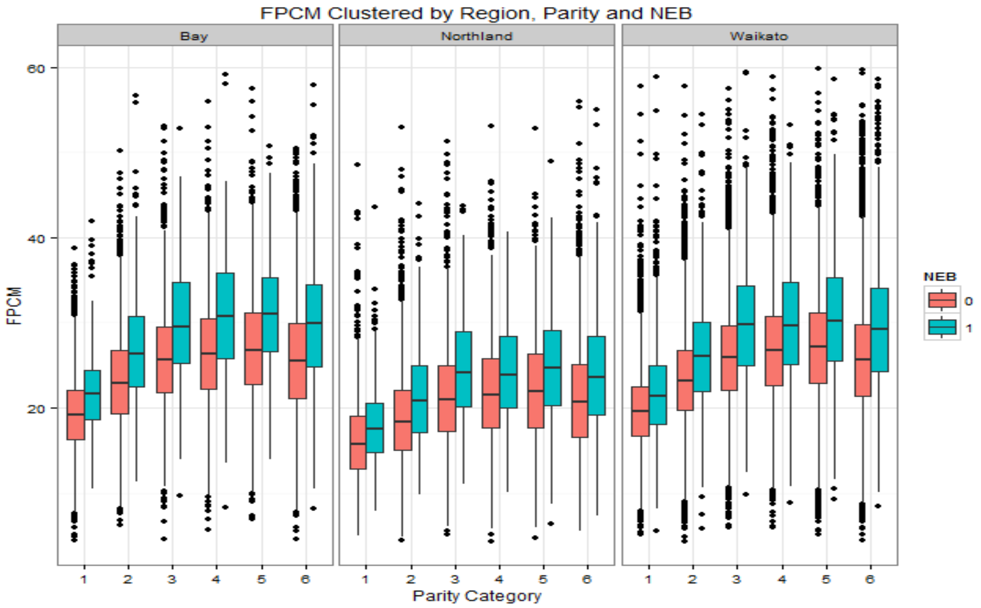

Figure 2.11 Boxplots of FPCM clustered by Region, Parity and NEB. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values.

The boxplots in Figure 2.12 show milk production trends in relation to NEB, subclinical mastitis and parity. Animals that had subclinical mastitis had generally low milk production across all parity categories compared to those that did not. This would therefore suggest that subclinical mastitis (high SCC) lowered milk production.

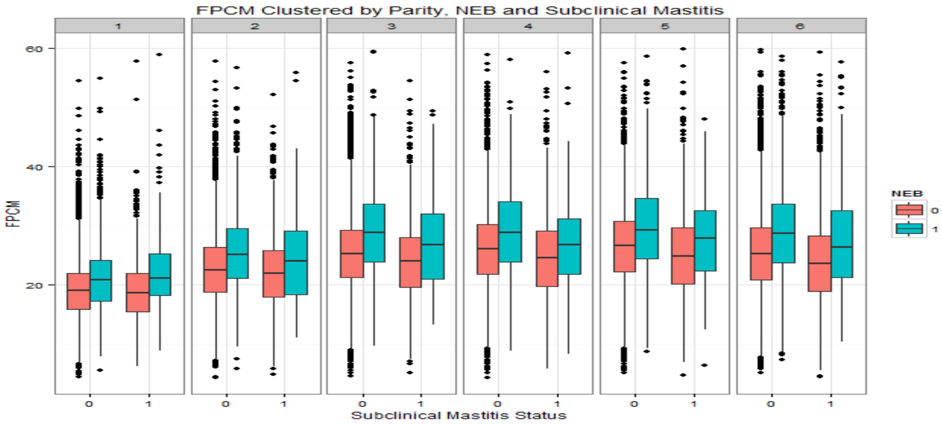

Figure 2.12 Box plot of FPCM clustered by Parity, NEB and Subclinical Mastitis. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values.

Boxplots of fat % versus parity and test number indicated that the first test had the lowest average fat % in milk compared to the other subsequent tests (Figure 2.13). There was a consistent increase in fat % from tests one to four with means of 4.48, 4.65, 4.88 and 5.27, respectively. Milk protein % indicated little variability among the different testing times except for an increase observed for test four (Figure 2.14). The means for protein % for tests one to four were 3.56, 3.55, 3.64 and 3.86 respectively. The average FPR was lowest for test one and was increasing with subsequent test number and was highest for test four as shown in boxplots in Figure 2.15, with means of 1.26, 1.31, 1.34 and 1.36, respectively. Comparison of FPR on boxplots showed no obvious differences among different parity categories within each specific test number except for parity one cows during first test which had a slight higher FPR than the rest.

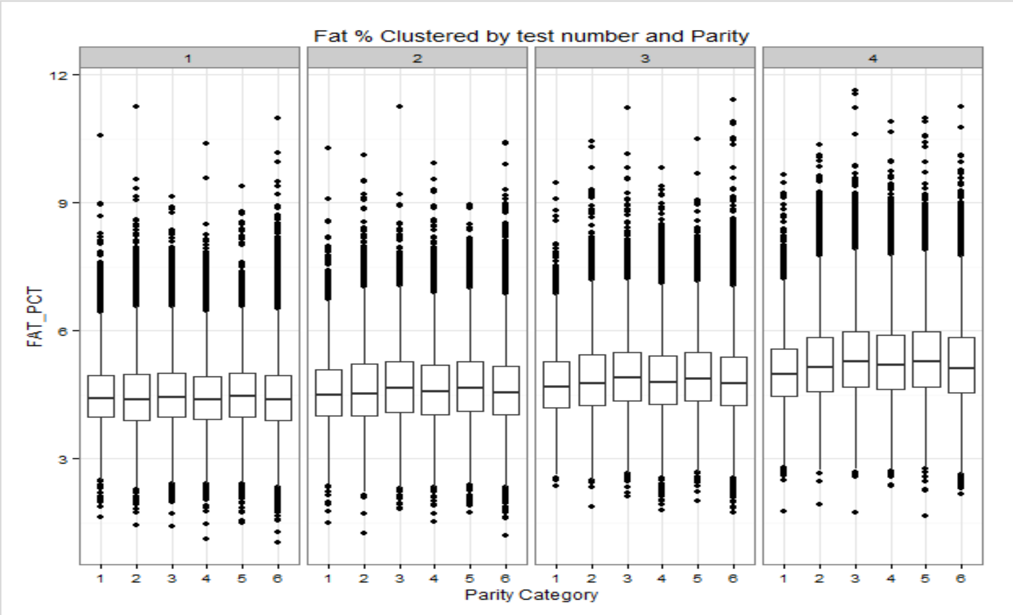

Figure 2.13 Boxplot of Fat % clustered by Parity and test number. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values.

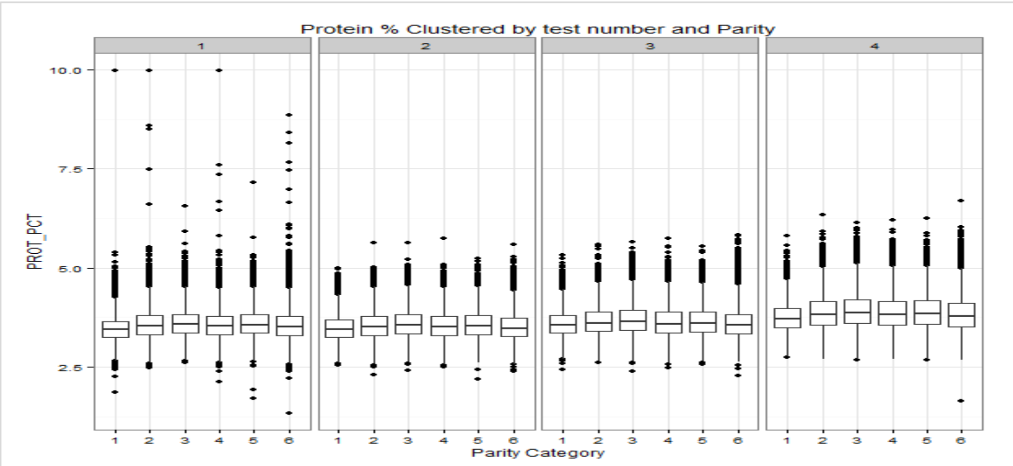

Figure 2.14 Boxplot of Protein % clustered by Parity and test number. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values.

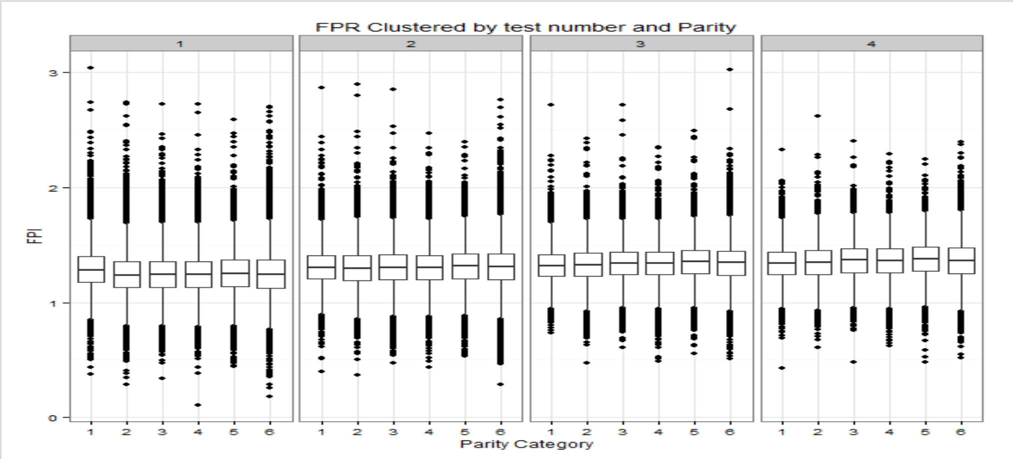

Figure 2.15 Boxplot of the fat-protein ratio (FPR=NEB) clustered by Parity and test number. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values.

The mean SCC for first test (185,000/ml) was lower than the overall mean involving all the four tests (198,000/ml). With exception of parity one cows, the mean SCC for animals in NEB tended to be higher than those that were not. The trend was similar when the log of SCC (logSCC) was compared using boxplots as shown in figure 2.16 which were clustered according to NEB, parity and region. Animals in NEB had generally higher logSCC across all parity categories for the Waikato region, but had variations in the other two regions. Only animals of parities 2 and 6 from the Bay of Plenty and parities 2, 5 and 6 from the Northland region in NEB had higher SCC. Animals in parity one were seen to have the lowest somatic cell count across all regions. It was observed that that logSCC was increasing with increase in parity implying that older animals had generally higher counts compared to the younger ones. The Bay of Plenty had the highest logSCC followed by Northland and then the Waikato region. The median counts for the logSCC against NEB status were observed to be similar across many parity categories within each region and this was more pronounced for the Waikato region.

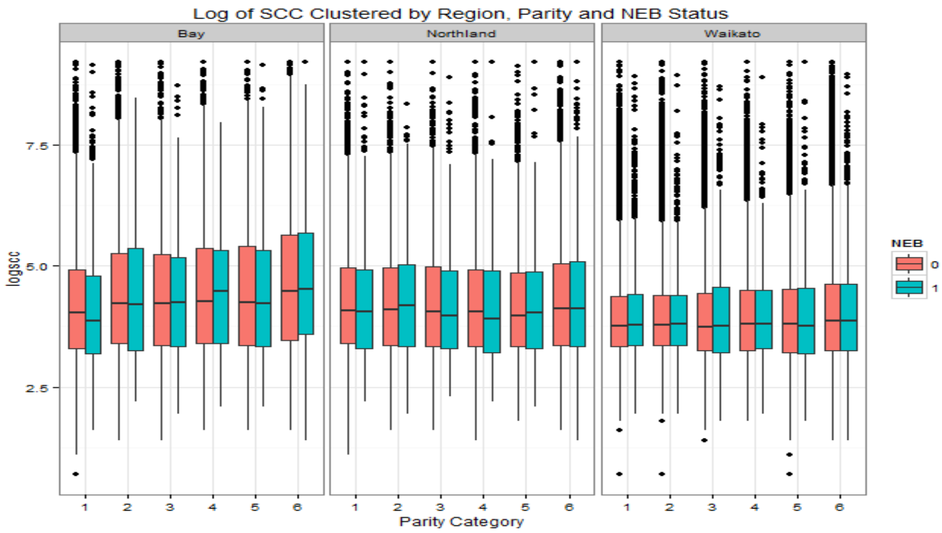

Figure 2.16 Boxplots of log of somatic cell count clustered by Parity, NEB and Region. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values.

The logSCC was lowest for herd test one and generally increasing with each subsequent test as shown in Figure 2.17. Animals in NEB during herd test one had higher logSCC across all parity category but was not the case for the other subsequent herd tests. The logSCC during herd tests two to four was lower for animals in NEB except for parity four during test two, parities five and six during test three, and parity six during test number four.

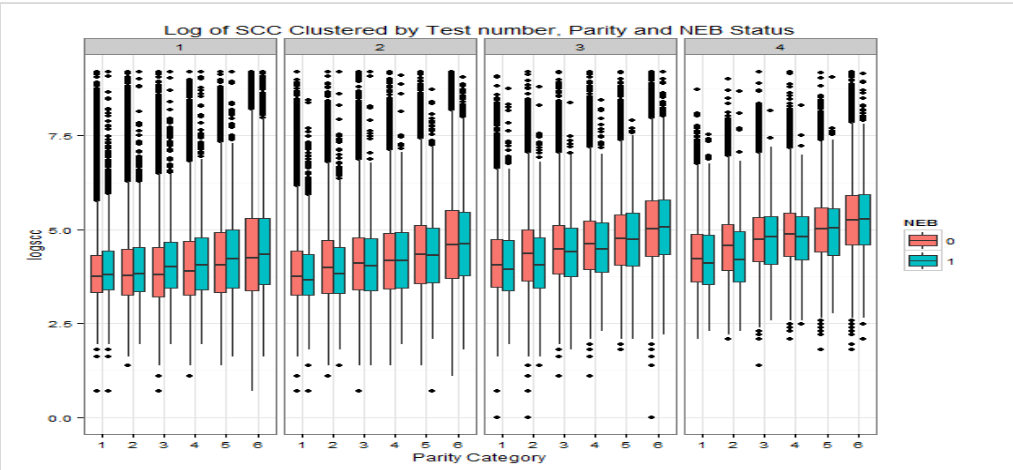

Figure 2.17 Boxplots of logSCC clustered by test number, NEB and parity. Contained in the box is the median, limits of the box indicate the 25th and 75th percentile, vertical lines show typical data range and solid circles show extreme values

2.3.2 Results of multivariate analyses.

2.3.2.1 FPCM

The results of the linear mixed regression model displaying the coefficient estimates, standard errors (SE) the 95% confidence intervals (CI) and the P values are shown in table 2.10. The reference group in the model was parity one non-pregnant Friesian cows from the Bay of Plenty in DIM stage one that were not in either NEB or subclinical mastitis.

Table 2.11~~10~~ Results from the linear mixed effects regression model for the effect of NEB on FPCM (kg/day) adjusted for several covariate.

	Estimate	SE	Lower 95% CI	Upper 95% CI	P-value
Intercept	19.82	0.389	19.06	20.58	<0.001
Negative energy balance	2.97	0.081	2.82	3.13	<0.001
Subclinical mastitis	-0.68	0.043	-0.77	-0.60	<0.001
DIM stage 1	*Reference*				
DIM stage 2	-1.52	0.032	-1.58	-1.46	<0.001
DIM stage 3	-5.11	0.137	-5.37	-4.84	<0.001
NEB*DIM stage 2	0.27	0.101	0.07	0.47	0.007
NEB*DIM stage 3	-1.45	0.246	-1.93	-0.97	<0.001
Herd Size	0.001	0.001	0.000	0.003	0.040
Parity 1	*Reference*				
Parity 2	3.39	0.048	3.30	3.48	<0.001
Parity 3	6.08	0.050	5.98	6.17	<0.001
Parity 4	6.87	0.051	6.77	6.97	<0.001
Parity 5	7.34	0.054	7.23	7.44	<0.001
Parity 6	6.17	0.043	6.09	6.26	<0.001
Friesian Breed	*Reference*				
Jersey breed	-0.23	0.062	-0.35	-0.11	<0.001
Cross-breed	0.55	0.039	0.48	0.63	<0.001
Bay of Plenty region	*Reference*				
Northland region	-4.07	0.421	-4.90	-3.24	<0.001
Waikato region	0.07	0.344	-0.60	0.75	0.828

From the model results, it could be seen that all the variables in the model were significant in predicting FPCM except for the region dummy variable for the Waikato. The model predicted an overall average FPCM of 19.82 kg per cow per day for the reference group. Animals in NEB were observed to have a significantly higher milk production by 2.97 kg in the reference group. This prediction was comparable to the boxplots that were developed under descriptive statistics as shown in Figures 2.9 to 2.11 in which animals in NEB were observed to have a higher production.

Subclinical mastitis significantly reduced FPCM by 0.68 kg (P < 0.001). An increase in DIM resulted in a statistically significant reduction in milk production where animals in DIM stage 2 had an average reduction of 1.51 kg and those in DIM stage 3 had an average reduction of 5.11 kg. This suggested that animals in stage 1 produced more milk than those in other DIM stages. The interaction between NEB and DIM stage showed significant effect in which production was 0.27 kg higher in DIM stage 2 than DIM stage 1 and decreased by 1.45 kg in DIM stage 3 for NEB cows. An increase in herd size resulted in a small increase in FPCM.

There was a linear increase in the milk production with increasing parity from parities 2 to 5 after which production dropped by 1.17 kg for parity 6 compared to parity five. The Jersey cows produced 0.23Kg less milk compared to Friesian, whereas the cross-breed cows produced 0.55 kg more milk. There was no significance difference in milk production between animals from Bay of Plenty and Waikato regions even though the later produced about 100g more on average, but animals from Northland produced significantly low milk by 4.07 kg.

The standard deviation for the herd random effect was 3.09, a variance of 9.57, and the residual standard deviation was 4.92, a variance of 24.19, resulting in a total variance of 33.76. The residual variance of 24.19 was associated with cow level factors other than those in the model. This therefore showed that there was more variability in milk production between cows than between herds. The proportion of variance at herd level was 0.284 given by 9.57/(9.57+24.19), which is also the intra-class correlation coefficient (ICC) measuring the production similarity of cows within a herd. The ICC was strong (>0.2), and therefore could be said that the herds in the dataset had comparable production. In this case the 95% of the herd average production was between 12.2 kg (18.3-(1.96*3.09)) and 24.4 kg (18.3 + (1.96*3.09)).

The likelihood ratio test output had a significant P value (<0.001) which showed that region was a significant variable in the model. The AIC equally reduced from 787,857 to 787,730 after region was put in the model. This could also be visualised from the boxplots, which reviewed differences in milk production amongst the three regions with the Northland producing significantly less milk at animal level compared to other two.

Analysis of model fit was done by inspecting two residues plots as shown in Figure 2.18. Histogram of the residuals was normally distributed around zero and the normal quartile plot did not review deviation from normality which indicated the assumptions of modelling were satisfied.

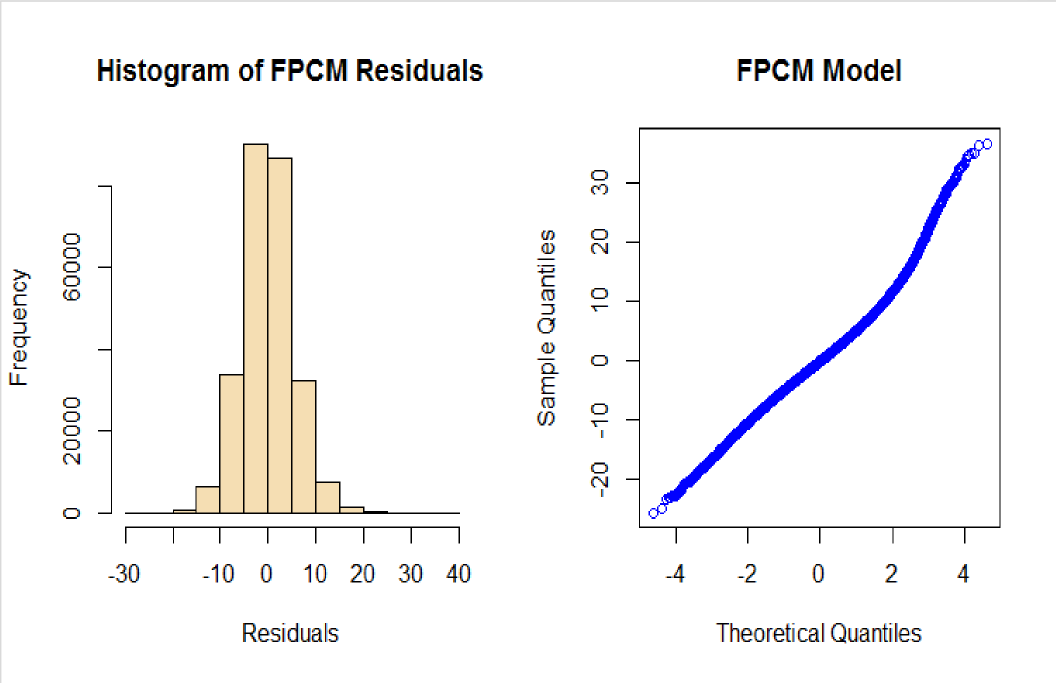

Figure 2.18 FPCM model diagnostics showing residuals histogram and residual normal quartile plot.

2.3.2.2 Model on somatic cell count prediction (Transformed to log scale).

A linear mixed model was performed to predict natural logSCC (x 10^3) with several predictors with herd included as a random effect. Model outputs are shown in table 2.12. The reference group was Friesian cows from the Bay of Plenty in parity one and DIM stage 1 that were not in NEB.

Table 2.12~~11~~ Effect NEB on logSCC adjusted for several covariates

	Estimate	SE	Lower 95% CI	Upper 95% CI	P-value
Intercept	4.091	0.048	3.997	4.185	<0.0001
Negative Energy Balance	0.058	0.018	0.023	0.093	0.0013
FPCM	-0.008	0.001	-0.009	-0.007	<0.0001
DIM stage 1	Reference.				
DIM stage 2	-0.132	0.007	-0.146	-0.118	<0.0001
DIM stage 3	0.187	0.03	0.129	0.245	<0.0001
Calving order	Reference.				
Calving order 2	0.009	0.007	-0.005	0.024	0.2151
Calving order 3	0.017	0.007	0.002	0.031	0.0266
Herd size	0.0004	0.0001	0.0001	0.001	<0.0001
Parity 1	Reference.				
Parity 2	0.043	0.011	0.022	0.064	0.0001
Parity 3	0.105	0.012	0.082	0.128	<0.0001
Parity 4	0.203	0.012	0.179	0.227	<0.0001
Parity 5	0.355	0.013	0.330	0.380	<0.0001

Parity 6	0.556	0.01	0.536	0.576	<0.0001
Friesian Breed	Reference.				
Jersey Breed	0.009	0.013	-0.017	0.036	0.4846
Cross- Breed	-0.026	0.009	-0.043	-0.010	0.0021
Bay of Plenty	Reference.				
Northland Region	0.154	0.05	0.056	0.251	0.0021
Waikato Region	-0.137	0.04	-0.216	-0.058	0.0007
NEB:DIM stage 2	0.045	0.022	0.001	0.089	0.043
NEB:DIM stage 3	-0.019	0.054	-0.125	0.087	0.7276

The model predicted an average logSCC of 4.082 for the reference group equivalent to 59,000 cells/ml. NEB significantly increased the logSCC by 0.058, equivalent to an increase from, for example, 240,000 to 255,000 SCC/ml. Higher milk production was associated with a lower logSCC by 0.008 per kg FPCM, equivalent to 3,500 SCC/ml for a standard deviation change in FPCM. This would follow that animals that were high producers with FPCM of 55 kg for example, would have a reduction in logSCC by 0.33, equivalent to 1,400 SCC/ml keeping other variables and the fixed effect constant. Compared to DIM stage 1, the logSCC in the DIM stage 2 was lower by 0.132 (1,206 SCC/ml) but higher in DIM stage 3 by 0.187 (1,141 SCC/ml) keeping other variables constant. Animals that calved in the second and third calving orders had significantly higher logSCC compared to those that calved in the first order. Herd size had a small effect though significant with each unit increase in herd size (1,000 SCC/ml), which could be due to contribution of large herds of which the largest had 1,573 cows. The logSCC was higher for each increase in parity category of the cows where parity 2 was higher by 0.043 (1,044 SCC/ml) and parity 6 by 0.556 (1,743 SCC/ml) compared to parity 1 keeping other variables constant. This indicated that old animals had higher SCC, putting them at higher risk of subclinical mastitis and/or clinical mastitis. The Jersey had a higher logSCC (1,010 SCC/ml) but the cross-breed had a lower logSCC (1,000 SCC/ml) compared to the Friesian keeping other variables and the fixed effect constant. There was a significant difference among the regions where the Waikato had a lower logSCC by 0.137 (1,150 SCC/ml) while the Northland had a higher logSCC by 0.154 (1,160 SCC/ml) compared to the Bay of plenty. Interaction of NEB and DIM stage 2 had a significant effect which increased the logSCC but not with DIM stage 3.

The variance due to the herd random effect was 0.128 (0.358^2) and residual variance was 1.121 (1.097^2) giving a total variance of 1.249. The ICC at herd level was weak (0.10), indicating a reasonably big variation of logSCC among herds. With the model intercept at 4.082, the 95% overall means of logSCC for the reference group means would be expected to lie between 3.38 (30,000 SCC/ml) and 4.78 (120,000 SCC/ml).

The analysis of model fit through residue plots is shown in Figure 2.19. The plot of standardised residuals against fitted values did not show any pattern, the histogram of the residuals was normally distributed around zero and the quartile normal plot showed no obvious deviation suggesting satisfaction of model assumptions.

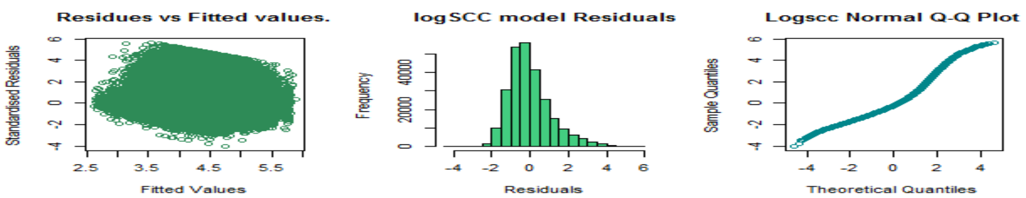

Figure 2.19 Model fit diagnostics for the logSCC model showing residual plots.

2.3.2.3 Model on subclinical mastitis.

A generalised mixed logistic model was performed to analysis of the relationship between subclinical mastitis and several predictors. The results from the model output are shown in table 2.13.

Table 2.13~~12~~ Effect of NEB on subclinical mastitis adjusted for several covariates.

	Odds ratio	SE	Lower 95% CI	Upper 95% CI	P-value
Intercept	0.125	0.069	0.109	0.143	<0.001
NEB	1.070	0.032	1.005	1.139	0.035
FPCM	0.975	0.002	0.972	0.978	<0.001
DIM stage 1	*Reference*				
DIM stage 2	0.755	0.019	0.727	0.784	<0.001
DIM stage 3	1.238	0.064	1.091	1.405	0.001
Calving order 1	Reference				
Calving order 2	1.025	0.021	0.983	1.069	0.24
Calving order 3	1.045	0.021	1.002	1.089	0.041
Parity 1	*Reference*				
Parity 2	1.317	0.039	1.220	1.422	<0.001
Parity 3	1.664	0.040	1.540	1.799	<0.001
Parity 4	2.285	0.039	2.115	2.467	<0.001
Parity 5	3.097	0.039	2.870	3.342	<0.001
Parity 6	4.825	0.033	4.528	5.143	<0.001
Friesian	*Reference*				

Jersey breed	0.884	0.036	0.823	0.949	0.001
Cross- breed	0.933	0.024	0.890	0.978	0.004
Bay of Plenty	*Reference*				
Northland Region	1.016	0.074	0.878	1.174	0.834
Waikato Region	0.789	0.061	0.701	0.889	<0.001

NEB significantly increased the occurrence of subclinical mastitis according to the model prediction with an odds ratio of 1.07 (7% increase, P = 0.035). Higher FPCM was associated with a lower probability of high SCC in milk (p<0.001) with an odds ratio of 0.975 implying that high producing cows were less likely to get subclinical mastitis, which however could as well be a dilution effect. The risk of subclinical mastitis was different between the two DIM stages. Compared to DIM stage 1, DIM stage 2 was associated with a lower risk (OR = 0.755) as opposed to stage 3 which had an increased the risk (OR = 1.238). There was no difference in risk of subclinical mastitis between animals that calved early and those calving at peak time. However calving relatively late increased the risk. The risk of subclinical mastitis increased with age: the odds ratio was highest for parity 6 (4.825) compared to parity 2 (1.317). Cases of subclinical mastitis were lower in both the Jersey and cross-breed compared to the Friesian which had odds ratios of 0.884 and 0.933 respectively. The Waikato region had less cases of subclinical mastitis compared to the Bay of Plenty with odds ratio of 0.789 while Northland was similar to Bay of Plenty.

The total variance of the random effect (herd) was 3.541 ($0.251+\pi^2/3$) giving a weak ICC of 0.071. This suggested a small variation or little clustering among different herds, meaning cases of subclinical mastitis were similar among different herds. Figure 2.20 shows the normal quartile plot of the random effect residuals which were within acceptable limits with a mean of 0.01 and range of 2.52.

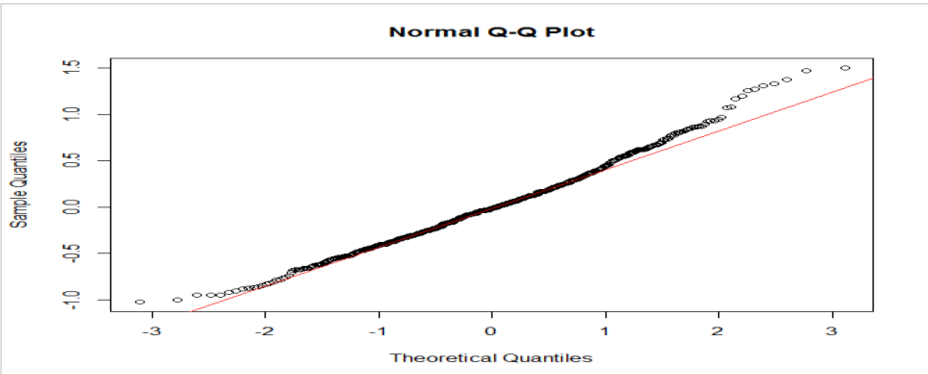

Figure 2.20 Random effects residual normal quartile plot

2.3.2.4 Model on pregnancy

A generalised mixed logistic model was performed to investigate the relationship between pregnancy and several predictors. The reference group was the Friesian cows in parity one from the Bay of Plenty that were not in state of NEB or subclinical mastitis. The results from the model are shown in table 16 and the intercept in this case is not meaningful.

Table 2.14~~13~~ Pregnancy model outputs showing the odds ratios, standard errors (SE), the 95% confidence intervals (CI) and the P values.

	Odds ratio	SE	Lower 95% CI	Upper 95% CI	P-value
Intercept	7.074	0.100	5.816	8.605	< 0.001
NEB	0.900	0.034	0.843	0.962	0.002
Subclinical mastitis	0.859	0.026	0.816	0.905	< 0.001
FPCM	1.017	0.002	1.014	1.021	< 0.001
Parity 1	*Reference*.				
Parity 2	0.951	0.034	0.889	1.016	0.137
Parity 3	0.971	0.037	0.903	1.043	0.420
Parity 4	0.800	0.037	0.744	0.861	< 0.001
Parity 5	0.674	0.038	0.626	0.725	< 0.001
Parity 6	0.497	0.030	0.469	0.528	< 0.001
Friesian Breed	*Reference*				
Jersey Breed	1.069	0.040	0.989	1.156	0.093
Cross-Breed	1.227	0.026	1.165	1.291	< 0.001
Bay of Plenty	*Reference*				
Northland Region	1.744	0.132	1.346	2.259	< 0.001
Waikato Region	1.294	0.107	1.049	1.597	0.016

The model predicted that both NEB and high SCC had negative effects on pregnancy with odds ratios of 0.900 and 0.859 respectively. Milk production was positively correlated with pregnancy suggesting that high milking cows were more likely to get pregnant (OR=1.017; p<0.001). The odds of getting pregnant were declining with increasing animal parity. For example, pregnancy rates of cows in parity 3 or 6 were only 0.971 or 0.497 times as high as of cows in first lactation. There was no significance difference in the pregnancy rates between Friesian and the Jersey cows (p=0.093), but cross-breeds had higher pregnancy rates than Friesian (OR=1.227; p<0.001). Pregnancy rates for both Northland and Waikato were higher compared to the Bay of Plenty, with odds ratios of 1.744 and 1.294, respectively. The total variance was 4.161 (0.8713+3.290), giving a strong ICC of 0.26, suggesting high clustering or similarities in the herds on pregnancy rates.

2.3.2.5 Model on culling for 2002

A generalised mixed logistic model was performed to investigate the relationship between culling in the 2002 and several predictors and only significant variables were included in the final model. The reference group was the Friesian cows in parity one that were not in NEB or subclinical mastitis. The results from the model are shown in Table 2.14.

Table 2.15~~14~~ Effect of NEB on culling for the 2002 breeding season

	Odds ratio	S.E	Lower 95% CI	Upper 95% CI	P Value
Intercept	0.700	0.082	0.596	0.821	<0.0001
Subclinical mastitis	1.609	0.023	1.539	1.681	<0.0001
NEB	1.362	0.030	1.285	1.444	<0.0001
FPCM	0.924	0.002	0.921	0.927	<0.0001
Parity 1	Reference.				
Parity 2	1.369	0.030	1.290	1.452	<0.0001
Parity 3	1.519	0.033	1.424	1.620	<0.0001
Parity 4	1.774	0.034	1.660	1.897	<0.0001
Parity 5	2.095	0.035	1.957	2.243	<0.0001
Parity 6	3.812	0.027	3.615	4.020	<0.0001
Friesian Breed	Reference.				
Jersey Breed	1.063	0.036	0.991	1.140	0.089.
Cross-Breed	0.923	0.023	0.882	0.966	<0.001

The model outputs predicted that subclinical mastitis and NEB both increased the risk of culling in the subsequent season of 2002 with the odds ratio of 1.609 (95% CI: 1.539 to 1.681) and 1.362 (95% CI: 1.285 to 1.444) respectively, (both p<0.001). Production on the other hand correlated negatively with culling; hence animals producing more milk were less likely to be culled.

As reasons for culling are failure to conceive, low production and various other diseases shown in Table 2.6, the risk of removal logically increased with parity. Compared to cows in parity one the odds of removal increased linearly in parities 2 to 6 with odds ratios 1.369, 1.1.519, 1.774, 2.095 and 3.812, respectively (all p<0.001). The cross-breed had less risk of being culled compared to the Friesian with an odds ratio of 0.92 but not with the Jersey breed which had a similar chance of being culled. There was no difference in the culling risks across different regions and the calving order of cows as a result these variables were dropped from the final model.

The total variance, 6.044 was given by a constant residual variance of 3.290 and the random effect variance of 2.76. This resulted to a strong ICC of 0.46 which suggested that culling was highly clustered in herds; hence, culling rates were herd specific even after adjusting for other variables shown in the model.

2.3.2.6 Survival analysis on PSM to conception interval.

The Kaplan-Meier curves were used to examine the effects of NEB, subclinical mastitis, breed and region on PSM to conception interval and are shown in figure 2.21. There was no apparent difference for the variables except for subclinical mastitis. Animals that had subclinical mastitis had a reduced PSM to conception interval. The log-rank test was significant for the three variables subclinical mastitis (p < 0.001), breed (p = 0.002) and region (P <0.001), but not for NEB (P = 0.153).Nevertheless, NEB was included in the cox-hazard proportional model because it was the primary variable of interest.

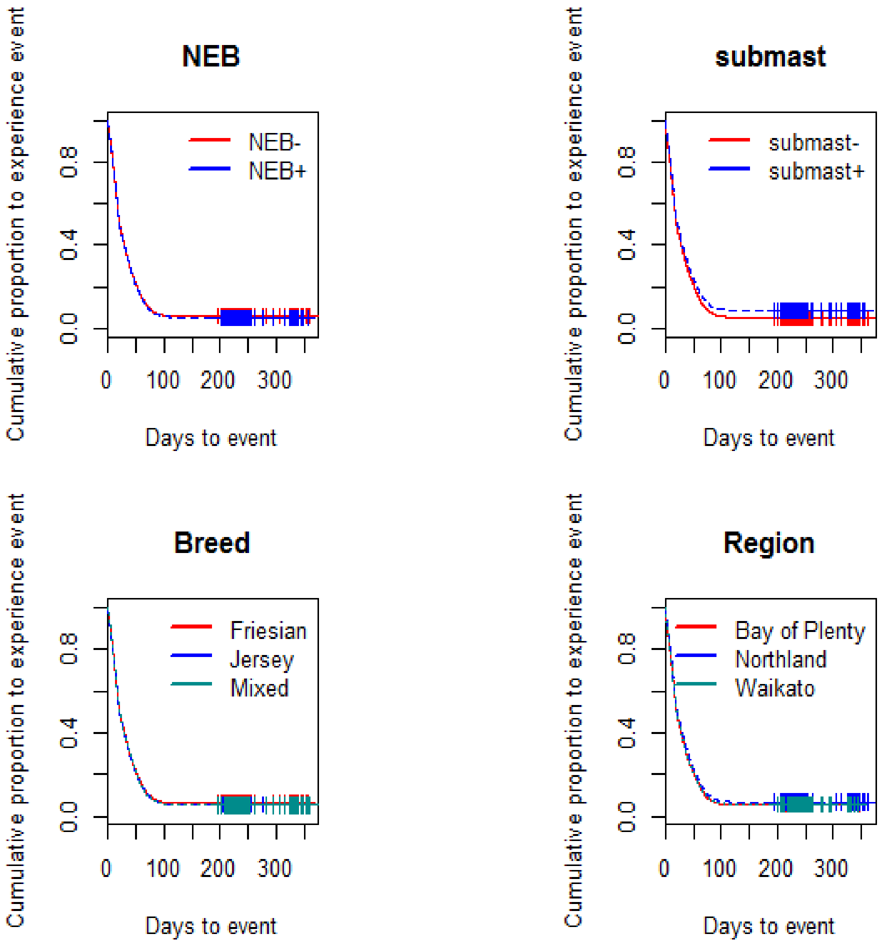

Figure 2.21 Kaplan-Meier curves for the effect NEB, subclinical mastitis, breed and region on PSM to conception.

The cox proportional hazards model results are shown in Table 2.15.

Table 2.16~~15~~ Cox proportional hazards model for PSM to conception interval for the 2001/02 season, showing the risk ratios, standard errors, 95% CI and the p value.

	Coefficient	SE	lower 95%	upper 95%	P value
NEB	1.006	0.012	0.983	1.031	0.610
Subclinical mastitis	0.897	0.010	0.879	0.916	< 0.001
NEB * subclinical mastitis	1.070	0.033	1.007	1.145	0.038
Parity 1	*Reference*				
Parity 2	1.035	0.011	1.023	1.068	0.001
Parity 3	1.105	0.011	1.092	1.142	< 0.001
Parity 4	1.084	0.012	1.073	1.124	< 0.001
Parity 5	1.070	0.012	1.053	1.105	< 0.001
Parity 6	0.968	0.010	0.958	0.995	0.001
Friesian	*Reference*				
Jersey	1.020	0.009	1.002	1.037	0.026
Cross-breed	1.023	0.008	1.009	1.041	0.004
Bay of Plenty	*Reference*				
Northland	0.935	0.011	0.912	0.951	< 0.001
Waikato	0.988	0.008	0.970	1.001	0.112

Comment [c3]: Plot predicted survival (1-cumHaz) for exposed/non-exposed cows, take a ruler and estimate how many days later exposed cows conceive at median survival (S=0.5).

NEB had no effect on PSM to conception interval holding other variables in the model constant, with a risk ratio of 1.006 (P = 0.610). Cows with subclinical mastitis had a lower PSM to conception with a risk ratio of 0.897 (P <0.001). The interaction between NEB and subclinical mastitis increased the PSM to conception interval by 7%. Parities 2 to 5 had higher PSM to conception interval compared to parity one but it was lower for parity 6 animals by 3.2%. Compared the Friesian, the Jersey and the Cross-breeds had increased risk ratios of getting pregnancy early by rates of 2% and 2.3%. The PSM to conception was similar between Waikato and the Bay of Plenty (P = 0.112) but it was lower for the Northland region by 6.5%.

2.4 Discussion

This study demonstrated the relationship of NEB with milk production, subclinical mastitis (high SCC), PSM to conception interval, pregnancy rates and the culling risk for the subsequent breeding season. The fat-protein ratio which was used to approximate NEB depended to a greater extent on the milk fat percentage than on the protein percentagebecause there was a consistent increase in percent-fat with each subsequent milk test (increasing DIM). This trendwas not as apparent for percent-protein contrary to findings from concentrate fed, high producing cows of in-door housing systems in the Northern hemisphere (Knop and Cernescu 2009). A total of 9.3% of cows were exposed to NEB and this was clearly lower than in, for example, high producing dairy cows in The Netherlands (Heuer, 2000). In our study, NEB impacted on outcomes like production, and

Comment [c4]: Not sure whether this is the correct reference, please check and add better ones here

Comment [c5]: We observed 25%, but you may have better refs reproduction performance which could potentially lead to substantial production loss if many cows were affected.

Average FPCM was different across different regions with overall averages of 20.52 kg, 19.95 kg and 20.92 kg for the Bay of Plenty, Northland and Waikato, respectively. These regional differences appeared to be higher than industry average milk production for the 2012 season of 17.66, 15.54 and 17.45 litres for the Bay of Plenty, Northland and Waikato region, respectively (DairyNZ.2012). However, if those figures were converted from volume (litre) to kilogramme, the milk production of the study herds was similar to industry averages. The incidence of subclinical mastitis and the logSCC were different between regions with a lower incidence in the Waikato than Bay of Plenty, and a higher incidence in Northland. A number of factors could have contributed to these differences, for example herd size, management and rainfall . Northland is located at higher latitude and therefore warmer with different rainfall patterns, which may have affected the growth and dry matter of grass. Regions may have different environmental micro-flora and hence different risk of mastitis. Pregnancy rates were higher in both Northland and Waikato regions than in the Bay of Plenty, but culling rates were similar in all the regions. The overall six week in-calf in this study was 68.8% which was lower than the New Zealand set target of 78% by 2016, but slightly higher than the 67% that was found in a study for herd data for 2009/10 and 2010/11 (Brownlie.2012). There were minor variations among different regions on the six weeks in-calf rate, that is 70.3%, 66.8%, 69.2% for the Bay of Plenty, Northland and Waikato, respectively, and overall pregnancy rate of 88.5%, which was slightly lower than the 90% pregnancy observed in a country wide study of 206 dairy herds (Brownlie.2012).

Milk production was higher with increasing parity with a linear increase seen up to parity five and then followed by a drop for parity six. Subclinical mastitis incidence was higher with increasing parity which has also been demonstrated by other studies as one of factors affecting SCC (Syridion *et al.*2012). Parity has also been reported to be a significant factor associated with diseases like mastitis, ovarian cysts, milk fever and ketosis in dairy animals (Uribe.1998), all of which are known to be production diseases with strong economic impart on dairy farming worldwide. Generally, young cows are less prone to production diseases with the exception of ketosis for which first calving cows had a higher risk than cows in lactations two or three (ref). Pregnancy rates also decreased with increasing parity. Infertility leads to involuntary culling. Culling rates as high as 33.7% due to infertility have been observed in dairy herds with 32% of first lactation heifers culled due to failure to conceive(Chiumia *et al.* 2013). A study in Irish cattle (Maher *et al.*2008) reported an average dairy herd culling rate of 21.3% while other studies have reported culling rates as higher as 34.58% (Stojic

Comment [c6]: Correct? A litre of milk weighs more than 1kg – see how much more it is and whether this statement is about right.

Comment [c7]: Avoid vague terms, e.g. climate is also part of environment, so this sounds like waffling.

Comment [c8]: Have you seen a reference about NEB in par1 cows? I remember this from my lit-review at the time.

Comment [c9]: Again too vague.

*et al.*2012). First calving cows were observed to contribute a share of 25.9% to culling in high producing herds, as opposed to 31.4% in low producing herds (Stojic *et al.*2013). A comparison of culling rates from this study and a study of other New Zealand dairy herds (Harris 1989) is shown in Table 2.16. For this study, culling rates were lowest at parities 3 and 4 as opposed to parity one in Harris study (1989). This former would be biologically plausible because cows could have been removed from the herds at parities one and two due to selecting higher milking cows to remain in the herd. From parity 5 onwards, culling rates increased with age. In systems with high milk production, cows are often selected after observing their performance in the first lactation, thereby screening for genetic merit by observing 1st lactation performance. This would explain the relatively high culling percentage of 1st calving cows in our study.

Comment [c10]: Did you limit the figures in table 2.16 to 365d culling rates? else cows would have aged into the next parity group.

Table 2.17~~16~~ Comparison of culling rates (%) of cows by parity category of this study with Harris (1989).

	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5	Parity 6+	Average
This Study	17.32%	17.50%	16.14%	16.65%	18.69%	28.63%	20.42%
Harris (1989)	13.43%	17.03%	18.18%	20.44%	22.59%	32.12%	20.63%

Jersey cows had on average a 230g lower production than Friesian cows, whereas cross-breeds produced 550g more, both differences being significant (P < 0.001). In a study of daily milk yield in these breeds and their crosses in South Africa Friesian produced more milk than cross-breeds while Jersey cows had the lowest milk yield, both in winter and spring (Nantapo and Muchenje.2013). Another study compared the performance of these breeds in United Kingdom and found that Friesian cows produced more milk, but milk fat and milk protein were highest in Jersey-Friesian crosses (Vance *et al.*2013). The breed specific production performances in this study were therefore comparable to other studies. Vance et.al (2013) reported that Jersey-Friesian crosses had fewer days to first observed heat, higher conception rate to first service and higher pregnancy at the end of the breeding season than pure Friesian cows. The findings in this study were similar, in that cross-breeds were more fecundant in that they had higher pregnancu rates (OR = 1.227; P<0.001) compared to the Friesian breed. The incidence of subclinical mastitis was lower for both, Jersey and cross-breeds compared to pure Friesian, whereas culling rates were higher for Jersey than Friesian cows.

The incidence of subclinical mastitis (high SCC) increased linearly with DIM , probably due to both, an increase in the rate of intra-mammary infections as well as decreasing milk volume (Petrovsky et al, 200..?). This was evident from the higher incidence rates between subsequent milk tests which were 12.5%, 13.6%, 18.3% and 23.1% for tests 1, 2, 3, and 4, respectively. Clinical mastitis contributed 3.15% of cows culled while 3.21% of animals were culled due to high somatic cell count. Since high SCC was prevalent in 15.9% of the cows and it was associated with reduced milk production, it is

Comment [c11]: A Northland study that showed this trend in clinical mastitis, except for a high rate in early lactation. Any other reference? You need to refer more to the literature when making suggestions like this.

likely that it contributed substantially to the 12.46% of cows removed due to low production. However, the recording of reasons for culling appeared to be poor as the majority of removals were for unknown causes.

NEB was observed to have a statistically significant effect on all evaluated responses, except for the PSM to conception interval. Cows in NEB produced 2.7 kg more milk at the first test than cows without NEB, and 2.4 kg more compared to the overall daily milk production. Dairy cows had higher production in early than mid- or late lactation which is often associated with body condition loss as animals mobilize body fat reserves to meet production demands (de Vries and Veerkamp 2000). This would predispose high producing cows to a higher risk of NEB which, in turn, would make them more susceptible to subclinical mastitis (high SCC) associated with potential milk production loss. This could then lead to losses of economic returns, possibly making prevention measures financially attractive.

Comment [c12]: Not sure what you mean here

The overall pregnancy rate from the data was at 88.6% with 51.7% conceiving at first service. The model predicted that both NEB and subclinical mastitis reduced pregnancy rates significantly. The modified Zhang and Yu method (Dwivedi *et al.*2013) was used to convert odds ratio to relative risk since this was a less biased estimate than the odds ratio. Relative risks were 0.944 and 0.925 for NEB and subclinical mastitis, respectively. NEB therefore reduced the pregnancy rate by 5.6% and subclinical mastitis by 7.5%. This effect was likely underestimated because NEB was approximated by the ratio of fat to protein percentage in milk, and hence could have been misclassified to some extent. involved with NEB from fat % and protein %, and if Assuming that this misclassification was the same in pregnant and non-pregnant cows, thus unrelated to the response (so called "non-differential misclassification"), the estimated impact was conservative, that is somewhat higher in reality. Poor fertility or failure to conceive is one the causes for involuntary culling, especially in the strictly seasonal reproductive management system in New Zealand. A study that looked at dairy cows removal reasons (Harris 1989) concluded that involuntary culling increased with age because of higher frequencies of poor fertility. Our study suggests that NEB contributes to some extent to infertility induced involuntary culling.

Comment [c13]: Where are the results of NEB on 1st service conception?

Comment [c14]: If average PR were 88.6% and RR=0.944 for NEB, then NEB reduced pregnancy rates from 88.6% down to 88.6 * 0.944 %. See the calculations in summary, and correct the stated estimates here. With only 9% NEB, the overall impact on herd pregnancy rates were not strongly affected.

Comment [c15]: Basically said above

NEB increased the chances of subclinical mastitis (high SCC) with a marginally significant odds ratio of 1.070 (P = 0.035). High SCC-cows are more prone to clinical mastitis, decreased milk yield, unfavourable changes in milk composition and increased culling rates (Lees and Lievaart.2013). A review of inter-relationships between NEB and udder health demonstrated evidence for patho-physiological mechanisms by which metabolic stress reduced udder defence, increased the risk of intra-mammary infection, clinical mastitis, antibiotic use and reduced milk production

(Suryasataporn et al 2000) There are other factors that have been associated with high SCC of which some could be management related and this could be exacerbated by NEB. Poor udder health is correlated with lower productivity, more antibiotic use and poor animal welfare, hence eventually important for the profitability of dairy farming and product safety and quality (Santman-Berends *et al.*2012).

The PSM to conception interval was not affected by NEB (RR = 1.006, p > 0.05). However, an interaction between NEB and subclinical mastitis increased the interval, suggesting that cows with both NEB and subclinical mastitis would conceive later than cows without either one or both conditions. The occurrence of both NEB and subclinical mastitis in the herd would eventually affect the generally productivity of the enterprise because affected animals would miss the peak seasonal pasture growth at calving, and possibly fail to achieve maximum milk yield. Since PSM is set in relation to pasture growth patterns, it is important for animals to calve at the right time so as progress normally in the subsequent breeding season and attain the 365 days breeding cycle. Compact calving before turnout of pasture in spring is an essential component of a pasture based dairy system to ensure maximum pasture utilization and eventually profitability (Herlihy *et al.*2011). It would therefore be of interest for farmers to take measures that would ensure conception is achieved within acceptable limits in relation to the PSM, which on some farms includes oestrus synchronization. The results of a six week in-calf rate of 68.8% stands in an unfavourable contrast to the 78% target set by the New Zealand dairy industry to be attained by 2016 (Brownlie.2012). The six week in-calf rate in this 2001/02 study was higher than the 67% observed by Brownlie (2012), thus fertility might have gradually decreased in this 10-year period. Other studies recommended that at least 80% of cows should calve within 6-8 weeks of the planned calving date (Dillon *et al.*1995) so that calving coincides with time of peak availability of grass from pasture. Our study demonstrated that NEB around calving may have small if any impact on calving patterns. A potentially greater impact of NEB may result if the fat-protein ratio prior to mating wouold be regressed on PSM to conception intervals.

Comment [c16]: Can you work out in a spreadsheet how lower the 6w-incalf rate was for NEB+SCM cows, and relatively how many were exposed in an average herd? The discussion becomes so much more worthwhile if you worked out the effects at the populatio0n level – epi-analysis does not end with a RR!

Comment [c17]: This is a long shot, try to avoid statements that force NEB to be seen as an important issue when that may not be the case. A marginally significant interaction like this would affect few cows only, so most likely not affect 'general productivity' to any notable extent.

2.5 Conclusion

High producing dairy cows were more likely to develop NEB, and this in turn tended to increase composite SCC. However, there was only a marginally significant effect that the increase in SCC also led to more cows with SCC above 250,000 cells/ml, a threshold above which cows may be classified as having intra-mammary infection, hence sub-clinical mastitis. NEB had relatively strong impacts on reduced final pregnancy rates and increased risk of removal. NEB had no significant effect on PSM to conception intervals but an interaction between NEB and subclinical mastitis increased the PSM to conception interval. Hence, the effect of NEB on final pregnancy may have occurred early in the mating period. This study explored relationships of NEB and various cow-level factors that inform hypotheses of future studies, for example about cause for cow wastage.

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Appendix

Table 2.18 Summaries of means, standard deviations and range (minimum min, maximum max) of study variables for overall observations tests (422,663 observations).

	Mean	SD	Median	Minimum	Maximum	Range	observations
Milk Fat	4.76	0.914	4.65	1.04	11.64	10.6	417325
Milk Protein	3.63	0.399	3.57	1.34	9.99	8.65	417325
SCC	198	517	74	1	9999	9998	417325
Subclinical mastitis	0.1602	0.367	0	0	1	1	417325
FPCM	20.4	6.3	19.6	4	59.8	55.8	417325
Days in milk	124	70	116	1	332	331	417325
DIM stage	2.4	0.698	2	1	3	2	417325
FPI	1.31	0.177	1.31	0.11	3.04	2.93	417325
Calve order	2	0.815	2	1	3	2	417325

Table 2.19 Summaries of means, standard deviations and range (minimum min, maximum max) of study variables for test one (129,960 cows)

	Mean	SD	Median	Minimum	Maximum	Range	Observations
Milk Fat	4.48	0.801	4.4	1.04	11.26	10.22	129947
Milk Protein	3.56	0.363	3.52	1.34	9.99	8.65	129947
SCC	186	586.2	50	2	9999	9997	129947
Subclinical mastitis	0.1254	0.331	0	0	1	1	129947
FPCM	24.1	6.8	23.8	4.3	59.8	55.5	129947
Days in milk	49	35	45	1	289	288	129947
DIM stage	2	0.56	2	1	3	2	129947
FPI	1.26	0.187	1.25	0.11	3.04	2.93	129947
Calve order	2.00	0.816	2	1	3	2	129947
NEB	0.0933	0.291	0	0	1	1	129947

Table 2.20 Summaries of means, standard deviations and range (minimum min, maximum max) of study variables for test two (120,297 cows)

	Mean	SD	Median	Min	Max	Range	Observations
Milk Fat	4.65	0.867	4.55	1.19	11.24	10.05	120286
Milk Protein	3.55	0.358	3.5	2.2	5.74	3.54	120286
SCC	183	506	64	2	9999	9997	120286
Subclinical mastitis	0.1363	0.343	0	0	1	1	120286
FPCM	20.2	5.7	19.7	4.2	59.1	54.9	120286
Days in milk	108	33	104	8	304	296	120286
DIM stage	2.2	0.432	2	1	3	2	120286
FPI	1.31	0.175	1.30	0.29	2.90	2.61	120286
Calve order	2.00	0.816	2	1	3	2	120286

Table 2.21 Summaries of means, standard deviations and range (minimum min, maximum max) of study variables for test three (129,960 cows).

	Mean	SD	Median	Min	Max	Range	Observations
Milk Fat	4.88	0.865	4.78	1.74	11.42	9.68	103101
Milk Protein	3.64	0.374	3.59	2.27	5.84	3.57	103101
SCC	213	500	97	1	9999	9998	103101
Subclinical mastitis	0.1832	0.387	0	0	1	1	103101
FPCM	18.2	4.7	17.9	4.1	58.3	54.2	103101
Days in milk	170	27	170	10	325	315	103101
FPI	1.34	0.158	1.34	0.47	3.02	2.55	103101

Table 2.22 Summaries of means, standard deviations and range (minimum min, maximum max) of study variables for test four (64,000 cows).

	Mean	SD	Median	Min	Max	Range	Observations
Milk Fat	5.27	0.987	5.15	1.66	11.64	9.98	63991
Milk Protein	3.86	0.444	3.8	1.64	6.7	5.06	63991
SCC	224	410	126	4	9999	9995	63991
Subclinical mastitis	0.2314	0.422	0	0	1	1	63991
FPCM	16.9	4.6	16.4	4.3	49.8	45.5	63991
Days in milk	221	25	222	85	320	235	63991
FPI	1.36	0.165	1.36	0.43	2.62	2.19	63991