Effects of cold stress on the submission and conception rates of New Zealand dairy cows

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Abstract

In the seasonal, pastoral-based dairy system of New Zealand, it is important for herds to achieve consistently high reproductive performance. A large amount of research has been conducted into the adverse effects of high temperatures on the fertility of dairy cows. However, similar studies quantifying the effects of cold conditions in grazing systems have not been carried out. In New Zealand, farmers regularly blame the weather for low submission rates and conception to artificial insemination. This is because cows that appear to be cycling well, prior to the planned start of mating (PSM), then appear to stop cycling during the mating season, commonly in conjunction with adverse weather conditions, such as exposure to short or extended periods of cold, wet and windy conditions. Therefore, the aim of the current study was to investigate the effects of cold, wet and windy conditions on the reproductive performance of dairy cows, in order to support or refute this farmer perception.

Calving, mating, lactation and pedigree records were provided by CRV Ambreed for the 2013 season, as well as calving records for the 2014 calving period, for 6664 cows from 20 herds throughout New Zealand. Climatic variables for the same time period were obtained from NIWA and used to calculate a cold stress index, which was analysed against the reproductive performance of each herd. The mean 21-day submission rate (SR21) of all 6664 cows was 77% and the mean 21-day in-calf rate (21d-ICR) was 45%. The average cold stress index (CSI) during the mating period was 967.4 $kJm^{-2}h^{-1}$.

The results from this study showed that there was a linear and quadratic effect of CSI on both SR21 and 21d-ICR, with an increase as the conditions began to cool, followed by a decrease in both submission and conception rates as the CSI reach levels above 1000 $kJm^{-2}h^{-1}$. This pattern may indicate a survival mechanism which allows the cows to produce more heat, but once CSI levels reach around 1000 $kJm^{-2}h^{-1}$, cows are no longer able to maintain body temperature and sustain production, and fertility suffers.

Further investigations should focus on the specific threshold level for the decline of SR21 and 21d-ICR, as well as the length of exposure and mechanisms which cause the decline in submission rates and conception rates.

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Chapter 1 : Introduction

The reproductive performance of dairy cows is an important aspect of dairy farming worldwide (Bonato and dos Santos, 2012). Selection for high milk production over the past three decades has allowed farmers to farm cows that produce more milk; however this selection has also been associated with a decline in fertility of the global dairy cow population (Statham, 2012). A high pregnancy rate is essential to ensure increased milk production, lower mating related costs and fewer health problems associated with calving and mating (Young, 1983). Therefore, determining the factors that may have an effect on the reproductive success of dairy cows, and how farmers can minimise these effects, will be critical for the improvement of reproductive efficiency in New Zealand dairy herds.

1.1 New Zealand dairy industry

In New Zealand, dairy farms operate with a single, seasonally-concentrated calving pattern (Macmillan and Moller, 1977). Therefore, to ensure that this pattern is continued, farmers need to maintain a 365 day interval between subsequent calvings. With the gestation length of a cow being around 282 days, this only leaves the farmer 83 days to get the cow pregnant, and accounting for an adequate postpartum anoestrus interval, this figure is reduced to around 41 days during which the cow is actively cycling (Boland, 1996). In a typical seasonal herd, breeding starts on a fixed date during spring and after this date, every cow which is detected in oestrus is bred (Grosshans et al., 1997). The latest industry target for analysing reproductive performance in the New Zealand dairy industry is the 6-week in calf rate (DairyNZ and LIC, 2014). This parameter calculates the proportion of cows that are pregnant within the first 6 weeks from the start of the mating period, which encompasses common artificial insemination (AI) periods used in New Zealand herds (DairyNZ and LIC, 2014). This parameter has been widely used recently as it is a good indicator of how compact the calving period is going to be.

For this study, the two main factors of reproduction that will be assessed are submission rate and in-calf rates. Submission rate in cows is defined as the total number of cows that are submitted for mating as a proportion of the total cows eligible for mating, during a specific time period (Statham, 2012). Conception rate for cows is defined as the total number of pregnancies as a proportion of the total number of inseminations during the mating period (Statham, 2012). Submission and conception rates, and the interaction between them account for the majority of the variation in 6-week in-calf rates between herds, and it is these two figures that the calculation of the 6-week in-calf rate are based upon (Morton, 2010). Therefore, it is important for farms to maximise both submission rates and conception rates in their herds, in order to achieve high reproductive performance.

1.2 Climatic Stress

Over the past 3 decades, there has been a large focus on the effect of climatic stress on the productive and reproductive performance of dairy cows, especially in tropical and sub-tropical environments (Statham, 2012). Climatic research is of particular importance in countries such as New Zealand and Australia, where cows are kept outdoors year-round, and can be exposed to extreme weather conditions which can impact the production and welfare of the animals (Bryant et al., 2010). The majority of research on climatic stress to date has been focused on heat stress, and these studies have conclusively shown a negative correlation between hot climatic conditions and reproductive performance in dairy cows (West et al., 2003; Nardone et al., 2006). It has been found that heat stress causes a change in the physiological functioning of the dairy cow, and manipulates hormonal secretions which cause a decline in fertility when cows are exposed to hot environments (Wolfenson et al., 1995).

However, although heat stress has a large impact in the dairy industry globally, research into the effect of cold conditions on reproductive performance has had very little attention (Bryant et al., 2007). Although there is evidence that cold conditions affect the reproductive function of dairy cows (Kilgour et al., 1977; Pennington et al., 1985), it is not well understood, with few relevant studies. A number of studies have demonstrated that lactating dairy cows are more tolerant of cold conditions than hot conditions (Mercier and Salisbury, 1947; Young, 1983). For example, studies in countries such as Canada and Europe have shown that during peak lactation, the lower critical temperature, which is the lowest temperature to which an animal can be exposed without being forced to increase its heat production, for dairy cows can be as low as -30°C in dry, still conditions (Mercier and Salisbury, 1947; Young, 1983). However, these studies are based on indoor dairy systems, with temperature being the only climatic variable that the cows are exposed to, and therefore, the results are not applicable to the outdoor systems utilised in New Zealand. Although we do not reach temperatures as far below zero as these countries, the combination of temperature, wind and rain have been shown to have an additive effect which can create a stressful environment for the cow (Bryant et al., 2007).

This thesis aims to determine whether there is a correlation between adverse weather events and submission or conception rates in New Zealand dairy herds, and if so, what the possible mechanisms are that may cause this effect. Furthermore, if a relationship is found, then the level of cold stress at which these effects begin to occur will be analysed, in order to determine the extent of cold stress that dairy cows are able to withstand, and during what conditions farmers need to be aware of reductions in submission and conception rates.

Chapter 2 : Literature Review

2.1 Introduction

Reproduction is an important aspect of dairy farming globally, and pregnancy rates are declining (Bonato and dos Santos, 2012). A high pregnancy rate is essential to ensure increased milk production, lower mating related costs and fewer health problems associated with calving and mating (Young, 1983). Therefore, determining the factors that may have an effect on the reproductive success of herds in different climatic areas, and how farmers can minimise these effects, will be critical for the improvement of reproductive efficiency in New Zealand dairy herds.

Farmers regularly blame the weather for low submission and conception rates to artificial insemination in New Zealand dairy herds (Burke, 2014). This is because cows that appear to be cycling well, prior to the planned start of mating (PSM), then appear to stop cycling during the mating season, commonly in conjunction with adverse weather conditions, such as exposure to short or extended periods of cold, wet and windy conditions (Burke, 2014).

Exposure of cows to a combination of cold, wet and windy conditions has been shown to increase plasma cortisol levels, which are known to be an indication of stress in the animal (Webster et al., 2008). An increase in the level of stress was shown, as animals exposed to cold, wet and windy conditions attempted to seek shade and shelter, in order to ameliorate the effects of the climate. However, in New Zealand, shelter is not always available for cows (Webster et al., 2008). Consequently, exposure to these conditions has resulted in cows increasing metabolic activity, in order to produce heat to maintain their body temperature (Ames, 1987). As a result of this, basal maintenance energy requirements are increased, and energy available for other processes such as milk production and reproduction are reduced (Broucek et al., 1991).

The purpose of this literature review is to build an understanding around the current knowledge of cold stress and its potential effects on reproduction in dairy cows and the mechanisms through which it may act. This review aims to examine the various factors that affect reproduction in the New Zealand dairy environment. The main focus will be on climate. After reviewing the literature, a hypothesis for the current research will be developed that will aim to provide an insight into an area that is not well defined or understood.

2.2 The reproductive cycle

2.2.1 Hormonal control of reproduction

The average oestrous cycle in cows lasts 21 days (18-24 days) (Bilodeau-Goeseels and Kastelic, 2003), and comprises of two to three waves of follicular growth (Figure 2.1). At the beginning of the cycle the hypothalamus releases gonadotrophin-releasing hormone (GnRH) which stimulates the pituitary gland to release pulsatile waves of follicle-stimulating hormone (FSH) and low levels of luteinising hormone (LH), as seen in Figure 2.1 (Eilts and Paccamonti, 2004). These hormones are important as they act on the follicles on the ovaries, allowing them to grow, and increasing their secretion of oestrogen (E2). This increase in E2 causes positive feedback on the anterior pituitary, as seen in Figure 2.2, causing an increase in secretion of both FSH and LH (Hafez, 1993). The increase in LH and FSH secretion caused by E2 stimulation can be seen in Figure 2.1, and it is this spike in LH that causes the dominant follicle to ovulate. Following ovulation, the remnants of this follicle undergo a number of functional and structural changes to form the corpus luteum (CL), which has an essential role in maintenance of pregnancy, as the CL produces progesterone (P4) (Bilodeau-Goeseels and Kastelic, 2003). The secretion of P4 from the CL is important as it causes a decrease in E2 and oxytocin receptors, which then limits the level of FSH and LH secreted by the hypothalamus. This allows time for fertilisation and establishment of pregnancy. In cows, the CL is the only source of P4, and therefore, if there is any malfunction, or decrease in P4 secreted by the CL, the pregnancy will be lost (Bilodeau-Goeseels and Kastelic, 2003).

When fertilisation is successful, the embryo will signal its presence to the dam, which results in the CL becoming insensitive to prostaglandin (PGF2α) (Bazer et al., 1993). Therefore, luteolysis is prevented and the CL continues to produce P4, maintaining the pregnancy (Bazer et al., 1993). However, if the ova does not become fertilised, then 16-17 days post-ovulation, P4 will begin to down regulate its own receptors, while up-regulating E2 and oxytocin receptors (Diskin and Sreenan, 1986). The CL will then begin to produce oxytocin, which signals the uterus to produce $PGF_2α$, causing luteolysis of the CL, allowing the dominant follicle of the current follicular phase to grow and ovulate, restarting the cycle (Hafez, 1993).

Figures 2.1 and 2.2 show a graphical representation of the hormone cycle that has been described. Figure 2.1 shows the changing hormone concentrations during the oestrous cycle. You can see here, the peak in oestrogen just prior to the large LH peak, which is key for the onset of oestrus behaviours, and the LH peak which causes ovulation to occur. Figure 2.2 shows the ovarian-pituitary axis, and the feedback loop that causes the release of each hormone involved. It is interesting as it denotes the different positive or negative feedback effects of the hormones, which helps to visualise why certain hormones are up- or down-regulated during certain periods of the cycle.

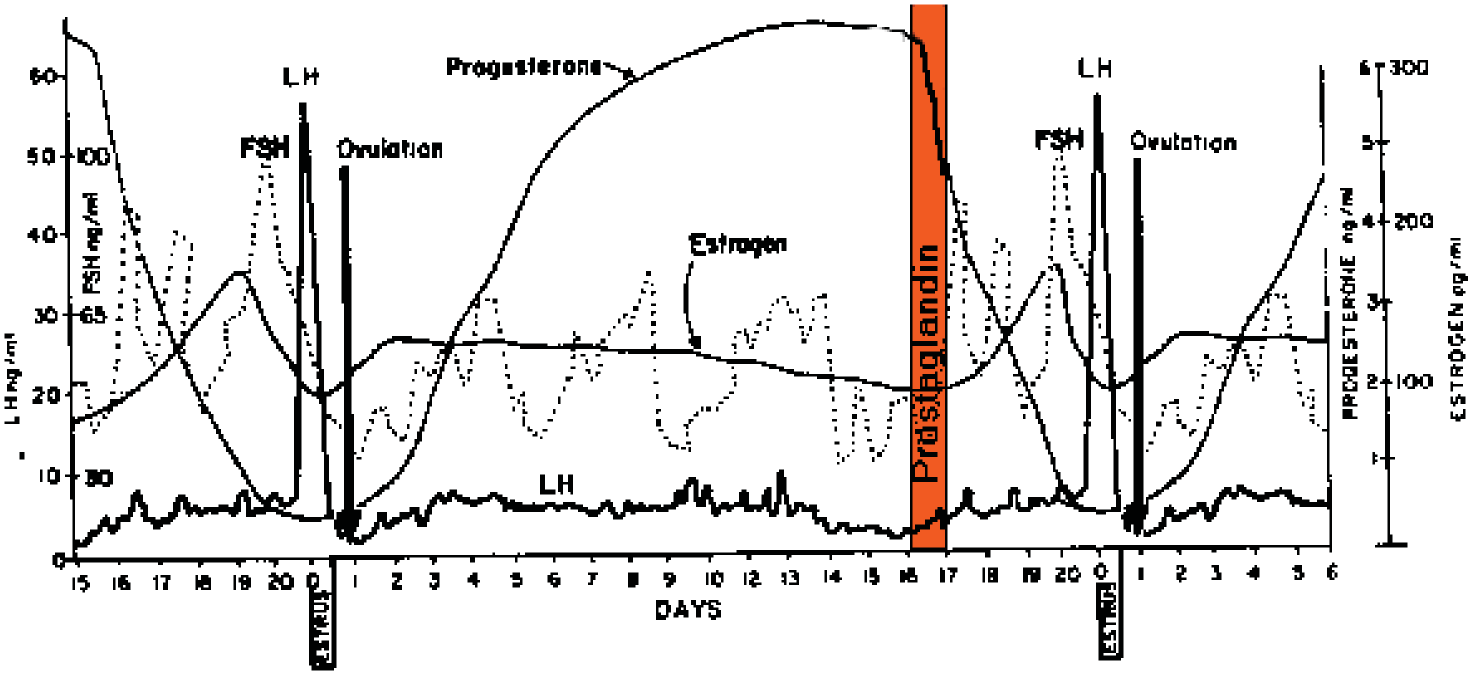


Figure 2.1: Graph denoting relative hormone concentration during the bovine oestrous cycle (Eilts and Paccamonti, 2004)

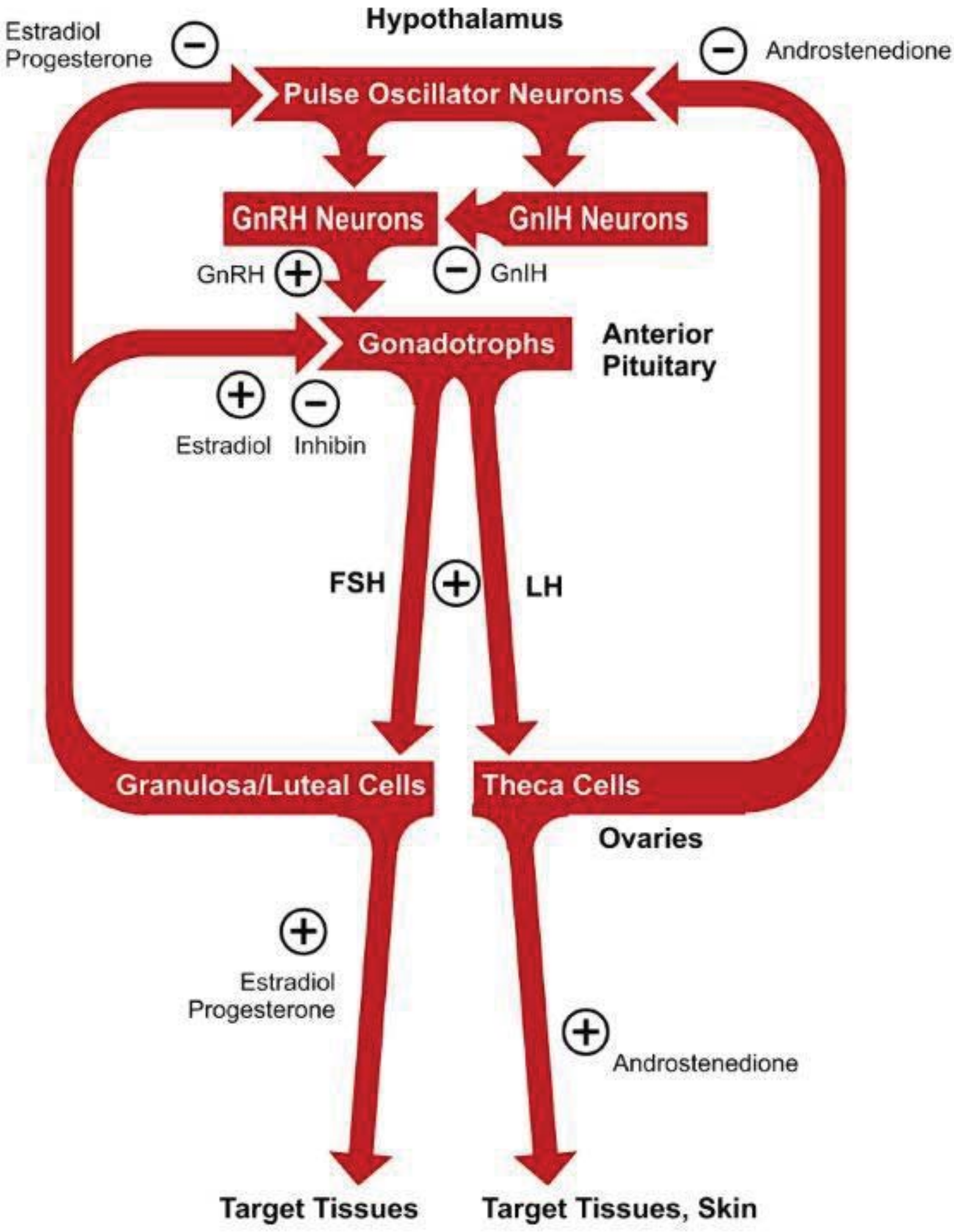


Figure 2.2: Graphical representation of hormones during the oestrous cycle (Peterson, 2014).

The concentration of P4 during specific periods of the cycle can lead to changes in the fertility of the cow (Zavy, 1994). Animals require prior exposure to P4 in order to display behavioural oestrus (Hafez, 1993; Zavy, 1994). Without this increase in P4 prior to ovulation, cows will ovulate without behavioural signals, such as mounting (Bilodeau-Goeseels and Kastelic, 2003). Additionally, this peak in P4 prior to ovulation also decreases the risk of double ovulations and increases the conception rate to AI (Ballarotti do Nascimento et al., 2013). Factors that decrease the production or concentration of P4 in the system can decrease the ability of the cow to conceive, or cause early embryonic death in cows that have conceived (Zavy, 1994).

Alterations to the circulating concentration of any hormone involved may alter the fertility of the cow, however, the majority of alterations that occur in the cycle result in changes to the concentration of P4. Circulating concentration of P4 in the cow is a balance between the rate of production and catabolism of P4 (Ballarotti do Nascimento et al., 2013). Production of P4 in the dairy cow is primarily from the CL, and the concentration is highly correlated to the size of the CL (Ballarotti do Nascimento et al., 2013). Catabolism of P4 is regulated by liver blood flow (Butler, 2000). Any mechanisms that cause an increase in liver blood flow, such as dry matter or energy intake and environmental changes can cause a decrease in circulating P4 concentrations.

2.2.2 Oestrus period

Oestrus is the period during the reproductive cycle when the animal is sexually receptive and is commonly referred to as the period when the animal is ‘on heat’ (Orihuela, 2000) (See Figure 2.1). During this period, the animal displays visual signs that she is on heat, such as standing to be mounted, a swollen pink vulva and mucus secretions, and increased physical activity (Orihuela, 2000). The intensity and duration of the oestrus period is highly variable between cows, commonly lasting between 10-18 hours, but periods as short as 3 hours have been recorded (Holman et al., 2011). Around 12-15 hours after the oestrus period has ceased, ovulation occurs, hence the oestrus period is used as an indication of when the cow should be bred, if pregnancy is to be achieved (White et al., 2002).

High rates of oestrus detection, with satisfactory accuracy have an important impact on the realisation of pregnancy in New Zealand dairy cows (Firk et al., 2002). This is because the majority of New Zealand dairy herds use AI during the first 6 week of the mating season (Macmillan and Watson, 1973). Unlike natural mating, where the bull detects when the cow is on heat and mates the cow, AI involves the farmer deciding when the cow should be submitted to be bred. Therefore, the oestrus period, and detection of it are extremely important (Orihuela, 2000). Positive impacts of increased oestrus detection on farm include improved insemination results, controlled calving interval and increased total pregnancy rates (Firk et al., 2002). However, undetected or falsely detected oestrus will result in wasted or ill-timed inseminations with consequent losses in income due to unexploited potential of milk and calf production (Firk et al., 2002).

2.2.3 Detection aids

Traditionally, oestrus detection has been performed using visual observation for behaviours such as mounting and restlessness (Firk et al., 2002). In addition to visual observation, farmers often incorporate further technical aids, such as heat mount detectors or tail paint to increase the frequency and accuracy of detection. However, these systems involve high labour requirements, with adequate staff training to ensure accuracy and efficiency of detection. As herd sizes have been continually increasing, the availability and ability of staff may not always be adequate to ensure efficient detection of oestrus (De Silva et al., 1981). With current advances in technology, automatic detection systems have been developed to help improve detection accuracy, while decreasing labour required, and therefore, decreasing human error (Firk et al., 2002).

Increased restlessness and activity are an indication of the cow being in oestrus (Firk et al., 2002). Many indoor systems, mainly in America and the UK, have changed from traditional methods of oestrus detection to using automated activity meters. Electronic pedometers can either be attached to the neck or leg, and continuously record activity (Firk et al., 2002). Changes in activity during oestrus are detected by comparing current activity levels to the activity of previous recorded periods which are associated with normal, non-oestrus behaviour (Firk et al., 2002). An advantage of this system is that farmers can set their own activity threshold which indicates the cow being in oestrus (Brehme et al., 2008). As activity increases have been reported between 30 and 393% higher than regular activity (Brehme et al., 2008), this system allows farmers to determine the average increase in their own system that will allow for high detection, with low false detections. In trials, this system has been shown to have an expected detection rate of 91%, in combination with 8% falsely detected cows, however, each farm will attain different results, reflecting the activity threshold that is selected to determine oestrus (Firk et al., 2002).

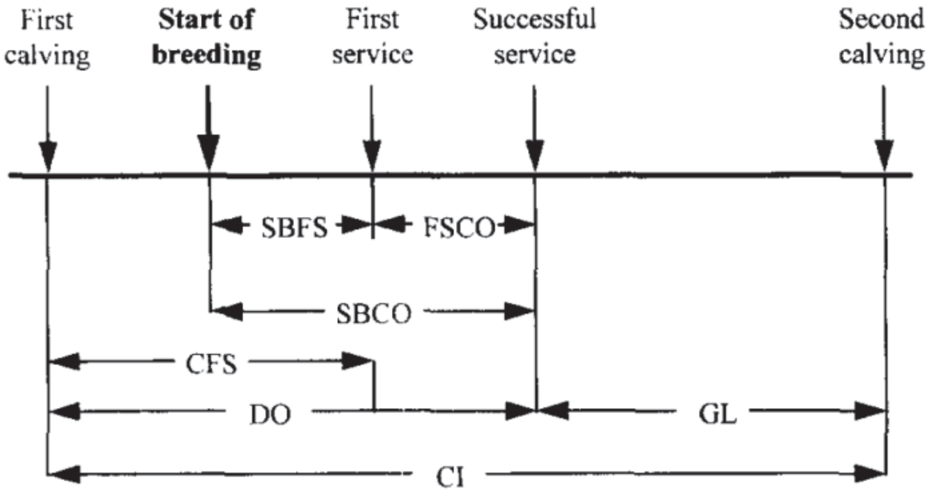
Another tool which has been developed and trialled on research farms is the biosensor. The biosensor is an automated sensor that determines the concentration of P4 circulating in the cow (Fir(Kappel et al., 2007)k et al., 2002). Progesterone concentrations are highly correlated with both oestrus and pregnancy and are commonly used in research trials as a tool for validating the accuracy of various detection methods (Firk et al., 2002). However, due to the difficulties of measurement of P4, either through milk or blood samples, it has not previously been practical as a tool for commercial farmers. A study in 2007 shows that the biosensor is able to accurately measure the level of P4 in milk within minutes of sampling, and can therefore determine periods of oestrus or pregnancy in cows (Kappel et al., 2007). However, this automated system has not been widely adopted, which may be due to a high cost associated with fitting this sensor into the milking parlour (Kappel et al., 2007). In addition to detecting oestrus, the biosensor can also be used as an aid to determine pregnancy, and therefore, serves as a bi-functional tool for farmers (Firk et al., 2002).

2.3 Measure of reproductive performance in seasonal grazing systems

Reproductive performance in dairy production systems is one of the most important elements of production efficiency (Grosshans et al., 1997). The main aim in seasonal herds is to achieve the highest pregnancy rate in the shortest time period (Macmillan and Watson, 1973). This allows the farmer to create a compact calving period for the following season. Therefore, effective reproduction management implies that within a determined period, i.e. the mating season, a pregnancy is achieved for each cow (Firk et al., 2002).

In New Zealand, dairy farms operate with a single, seasonally-concentrated calving pattern (Macmillan and Moller, 1977). Therefore, to ensure that this pattern is continued, farmers need to maintain a 365 day interval between subsequent calvings. With the gestation length of a cow being around 282 days, this only leaves the farmer 83 days to get the cow pregnant, and accounting for an adequate post-partum anoestrus interval, this figure is reduced to around 60 days (Boland, 1996). In a typical seasonal herd, breeding starts on a fixed date during spring and after this date, every cow which is detected in oestrus is bred (Grosshans et al., 1997). Therefore, by creating a compact calving period, the farmer will have more cows calving earlier in the calving season. This will allow the cows more time to start cycling prior to the start of mating, in comparison to cows that calve later in the season (Macmillan and Watson, 1973).

In dairy cows, there are a number of parameters used to define reproduction, and traditionally, these have been based around the calving date of the cow (Figure 2.3). These parameters include calving to first service (CFS), calving to conception, also known as days open (DO), first service until conception (FSCO), calving interval (CI) and age at calving (AC) (Grosshans et al., 1997).



SBFS: Interval from SB until first service; SBCO interval from SB until conception; CFS Interval from calving until first service; FSCO Interval from first service until conception; DO interval from calving until conception (days open); CI interval between consecutive calvings and GL gestation length.

Figure 2.3: Graphical Representation of the Fertility Traits (Grosshans et al., 1997).

However, these measures have been identified as unsuitable measures for seasonal calving herds (Morton, 2010). Long calving intervals do not necessarily reflect poor reproductive performance in seasonal calving herds, because cows that calved early in the calving period will have longer CFS and FSCO, than those cows that calved later in the period, which increases the CI. In addition, these parameters also do not account for cows that fail to conceive, or for a maximum number of inseminations, and therefore, does not portray the actual reproductive performance of the herd (Morton, 2010).

Subsequently, four traits that are unique to seasonal herds were developed. These include start of breeding until first service (SBFS), start of breeding until conception (SBCO) and the percentage of cows that conceived by either day 21 or 42 of the mating period (PR21, PR42) (Grosshans et al., 1997). Measures that relate to the start of breeding, rather than the calving date, are much more reliable in seasonal herds, as they identify actual herd performance, irrespective of the date the cow calved. The percentage of cows pregnant by days 21 and 42 (PR21 and PR42) are important in seasonal herds, as it is the aim to have as many cows pregnant as possible in a short time (Macmillan and Watson, 1973). These parameters allow the farmer to calculate the proportion of cows that are pregnant by specified intervals after the start of breeding. These parameters also take into account cows that have not conceived by a certain time period. However, these terms are no longer used in the modern NZ dairy system, but have been replaced with the 6-week in-calf rate, which is equivalent to PR42 (McDougall et al., 2014).

The two main drivers of the 6-week in-calf rate are submission and conception rates, and the target for New Zealand dairy farms is to reach a 6-week in-calf rate of 78%, which is calculated from industry set targets of a 90% 3-week submission rate and a targeted conception rate of 60% (Morton, 2010; DairyNZ and LIC, 2014). Submission rate in cows is defined as the total number of cows that are submitted for mating as a proportion of the total cows eligible for mating, during a specific time period (Statham, 2012). The most common time periods used are 21 days, as this is one complete cycle, or 24 days, to account for the variation in cycle length between individual cows. However, although the majority of cows have 21 day cycles, cycles ranging between 18-24 days are considered normal (Macmillan and Watson, 1973), and consequently, 3-week periods may be inadequate for all cows to be receptive to be bred. As a result, some studies have adopted a 4-week period to calculate submission rates (Macmillan and Watson, 1973). This longer period has been used for practical purposes, as it is long enough that all cows will be eligible to be bred, even if their cycle is longer than 21 days. Submission rate in cows can easily be improved on-farm through the use of exogenous hormones as part of a synchronisation scheme (Orihuela, 2000), or through automated detection aids, such as activity meters (Statham, 2012). However, these techniques may result in lower conception rates, due to the exogenous hormonal influences on follicular growth characteristics, which may lead to increased maturation and atresia of ova prior to ovulation (Statham, 2012).

Conception rate for cows is defined as the total number of pregnancies as a proportion of the total number of inseminations during the mating period (Statham, 2012). Therefore, the conception rate is determined by the accuracy of detection, and the efficiency of submission and insemination of the cow. The industry target for conception rates in New Zealand is 60% (DairyNZ and LIC,2014). The factors that can influence conception rate are extremely variable, and include disease, nutrition, semen quality, environmental stresses and social grouping (Nardone et al., 2006; Statham, 2012). These factors can decrease the likelihood of the cow to conceive, for example, lower oocyte quality as a result of poor nutrition (Orgal et al., 2012); or can cause embryonic death in cows that have conceived for example, negative energy balance can impact the progesterone levels in the uterus, which is essential for maintenance of pregnancy (Bilodeau-Goeseels and Kastelic, 2003).

In addition, many management factors, such as the interval between calving to the initiation of the breeding period, improved body condition score (BCS) going into lactation, and factors associated with milk protein concentration can affect both conception and submission rates, which shows that small improvements or changes to the farm system can help to improve reproductive performance (Morton, 2010).

2.4 Postpartum anoestrus interval

The post-partum anoestrous interval (PPAI) is the period after calving during which the cow does not recommence cyclic activity (Boland, 1996). This period occurs in order to ensure maximum survival of current offspring, and also allows time for involution of the uterus (Boland, 1996; Peter et al., 2009). Commonly, the PPAI lasts around 6 weeks (or 45 days) in dairy cows, but extended or prolonged PPAI is one of the major forms of infertility in New Zealand (McNaughton et al., 2003; Peter et al., 2009). The length of the PPAI is highly variable between cows, and can be affected by many factors, including disease, retained placenta, difficulties during calving and heat stress, however, it is most often associated with nutrition, or more commonly, negative energy balance (McDougall et al., 1995; Boland, 1996).

2.4.1 Negative energy balance

There are two key sources of energy for dairy cows, the first, and most common, being dietary energy supplied by feed, and when this is insufficient, cows will mobilise their body reserves in order to increase energy available for production (Garnsworthy and Topps, 1982). The level of energy in a given feed source will be dependent on the concentration and dry matter content of the feed. Dietary energy is expressed as mega joules of metabolisable energy per kilogram of dry matter (MJME/kgDM) (Nicol and Brookes, 2007). The metabolisable energy content of pastures and concentrate type feeds such as total mixed rations (TMR) are relatively similar (Kolver et al., 2002), however, pasture ME is more likely to fluctuate during the season as the climate changes (Nicol and Brookes, 2007). Therefore, total dietary intake of a cow will determine the amount of energy available for production traits such as milk production, growth and reproduction (Faverdin, 1999).

The initiation of lactation in dairy cows, as a result of calving, results in a large increase in energy demand (Nicol and Brookes, 2007). This increase in demand is often unable to be met by the cow, as required intakes are higher than the capacity of the cow to consume (Holmes et al., 2002c). Table 2.1 displays the change in energy demands between the dry period requiring an average dietary intake of 8.6 kgDM/day, to early lactation, requiring an average dietary intake of 18.9kg DM/day for a feed containing 11MJME/kgDM eaten (Nicol and Brookes, 2007). As this increase is over double the dry cow requirement, the cow is generally unable to consume the required amount of feed, and the cow is said to be in a state of negative energy balance (NEB) during early lactation (Butler, 2000).

Negative energy balance is commonly associated with a decrease in body condition as the cow will mobilise tissue to supplement dietary energy levels which allows for milk production to be maintained (Bilodeau-Goeseels and Kastelic, 2003). Due to the increased capacity of high genetic merit cows to produce milk, they tend to remain in a NEB for a longer period of lactation than lower genetic merit cows as reviewed by Holmes (1999). Additionally, high genetic merit cows have also been observed to have larger decreases in body condition, as shown in Table 2.2 (Holmes, 1999). This table shows that even at low stocking rates, where pasture allowances would be higher, high genetic merit cows still lose considerably more condition over the course of the lactation, in comparison to lower genetic merit cows (Holmes, 1999). This lower body condition occurs in order to maintain production levels, and is associated with a higher NEB, as reviewed by Veerkamp et al. (1995) (Roche et al., 2007).

Table 2.1: Daily metabolisable energy requirements of dairy cows (Nicol and Brookes, 2007)

	Growing heifer	Pregnant dry cow	Lactating cow	
			Early lactation	Late lactation
Feed(MJ ME/kgDM)	11	11	11	11
Live weight (kg)	250	500	550	450
Liveweight change (kg/day)	0.6	0.3	-0.5	0.5
Milk (L/day)	-	-	25	15
Milk fat (%)	-	-	4.5	5.0
Milk Protein (%)	-	-	3.5	3.7
Weeks from calving	-	4	-	-
Km walked/day	-	-	2	2
Km climbed/day	-	-	0.1	0.1
Calf birth weight (kg)	-	35	-	-
ME Requirements (MJ/day)				
Maintenance	36	57	61	52
Activity	-	-	6	5
Liveweight change	18	14	-14	19
Pregnancy	-	24	-	-
Lactation	-	-	155	99
Total	**54**	**94**	**208**	**175**
DM intake (kg/day)	4.9	8.6	18.9	15.9

Table 2.2: Performance of Jersey cows of high or low genetic merit, grazed on farmlets at different stocking rates (Holmes, 1999)

		High genetic merit (BI fat: 125)[1]				Low genetic merit (BI fat: 100)[1]			
Stocking rate (cows/ha)		2.77	3.26	3.75	4.28	3.75	4.28	4.76	5.26
Milksolids per cow (kg)		359	310	301	270	228	210	195	177
Milksolids per ha (kg)		995	1008	1127	1159	853	899	928	934
Days in milk		284	265	261	247	247	226	230	213
Liweight (kg) at:	Calving	393	390	368	364	361	340	329	331
	Dry-off	392	397	368	356	380	346	342	342
Body condition score at:	Calving	6.3	6.3	5.5	5.4	6.3	5.9	5.5	5.3
	Dry-off	4.6	4.9	4.0	3.9	6.1	5.0	4.8	4.9
Calculated feed eaten per cow (tDM)		4.0	3.7	3.6	3.4	3.2	3.0	2.9	2.8

[1] BI Fat is representative of the breeding index for milk fat production, with higher genetic merit cows producing more milk fat.

Due to the pastoral systems used in New Zealand, the ability of the cow to consume the required energy intake is further restricted. This is mainly due to the low dry matter content of pastures, which restricts the capacity of the cow to consume pasture, as the rumen fill occurs prior to energy intakes being met (Faverdin, 1999). Additionally, intakes may also be restricted by pasture characteristics such as height, density and quality, as these factors can influence the ability of the cow to harvest the pasture (Holmes et al., 2002c). Therefore, the cow's ability to reach her genetic potential for milk production under grazing systems is often lower than those of cows in a total mixed ration system, which is high in energy, so that the relative energy deficit, or NEB, may be minimised.

Table 2.3 demonstrates that with an increase in either live weight or milksolids production, the annual energy requirements of cows increase (Nicol and Brookes, 2007). High genetic merit cows have higher milk production, and often a heavier live weight also, and therefore, their energy demands will be even greater during early and peak lactation (Holmes, 1999). It is because of this increased negative energy balance that high genetic merit cows are required to mobilise a greater level of body reserves, in order to allow continued high milk production (Holmes, 1999).

Table 2.3: Annual metabolisable energy requirements (MJME/year) of dairy cows of different live weights and milksolids yield (Nicol and Brookes, 2007).

	Milksolids (kg MS/year)				
Live weight (kg)	300	350	400	450	500
			MJ ME/year		
350	40700	44600	48400	52300	56400
400	42900	46700	50600	54400	58300
450	45000	48900	52700	56600	60500
500	47100	50900	54800	58700	62600
550	49100	53000	56900	60800	64700
600	51100	55000	58900	62800	66700

2.4.2 Effects of the energy deficit on cow performance

A cow's productivity is determined largely by two key factors (Holmes et al., 2002a). The first is the management of the cow, including feeding, milking and health care, which is determined by the farmer (Holmes et al., 2002a). The second factor is the cows own capabilities, which is determined by her genetic merit or genetic abilities (Holmes et al., 2002a). The genetic ability of a cow is determined by the abilities of her parents, and increasing the average genetic merit of a herd is achieved through use of high genetic merit sires, in combination with effective culling decisions in which inferior females are culled and replaced with superior females (Holmes et al., 2002a).

Although increased milk production has increased economic returns for farmers, genetic selection for high milk production has been associated with negative effects on health, fertility and reproductive performance of the cow (Holmes et al., 2002a; Ferraretto et al., 2014). These health and fertility implications are related mainly to the extended negative energy balance of high genetic merit cows (Holmes, 1999). Increased production levels have been associated with an increase in the incidence of mastitis (Holmes et al., 2002b), as well as an increase in occurrence of metabolic diseases due to the prolonged period of negative energy balance (Holmes, 1999). These increased incidences of health disorders will result in decreased longevity in the herd, as well as an increase in health associated costs per cow.

Negative energy balance, or undernourishment in cows affects fertility in a range of ways. Firstly, it may result in an abnormal uterine environment, especially in relation to circulating hormone concentrations (Zavy, 1994; Butler, 2000). The physiological state of under-nutrition which occurs when the cow is in NEB suppresses LH secretion while also reducing ovarian responsiveness to LH, and prevents or delays ovulation, which would lead to an extended PPAI (Canfield and Butler, 1991; Beam and Butler, 1997). Additionally, this suppression of LH response occurs largely prior to the NEB nadir, and once the cows are returning towards positive energy balance, LH secretion increases and regular cycling is able to resume, however, conception rates may still be depressed until cows reach a positive energy balance (Beam and Butler, 1997). This delay in the increase of conception rates may be due to the effect of these changes on the follicular environment. Although LH is returning to normal levels and allowing ovulation to occur, follicular development can still be impaired by changes to the secretion of LH and FSH in relation to the normal secretion levels, leading to reduced quality of the follicles being ovulated (Beam and Butler, 1997). For example, beef cows that have been fed a low energy diet whilst nursing their calves were observed to have lower levels of FSH and LH (Bilodeau-Goeseels and Kastelic, 2003), as a result of lower energy availability, with these cows having reduced conception rates (Stagg et al., 1998; Butler, 2000). Consequently, high producing dairy cows tend to have a longer PPAI, due to the effects of the increased NEB on the hormonal environment of the reproductive axis, and this reduces the amount of time they have to get pregnant for the following season (Canfield and Butler, 1991; Boland, 1996; Beam and Butler, 1997).

The energy balance of lactating dairy cows during the early lactation period also has a regulatory role on the level of progesterone (P4) increase during the first 2-3 ovulatory cycles (Villa-Godoy et al., 1988). In cows with a NEB, circulating P4 concentrations are reduced, which resulted in fewer cows expressing oestrus before the first post-partum ovulation, and decreased conception rates, in relation to cows that had a more positive energy balance (Spicer et al., 1990). Table 2.4 shows the effect of loss of condition between calving and mating on the conception rate in high yielding dairy cows (Bourchier et al., 1987). It is clear that greater loss of body condition results in a reduced conception rate, and these findings are supported by additional studies which have reported significantly lower conception rates, and higher empty rates for high genetic merit cows under intensive grazing systems (Dillon and Buckley, 1998; Holmes, 1999). This decrease in conception rate may again be due to the changes in follicular development discussed earlier, but also has the added impact of low P4, which plays a critical role in the establishment and maintenance of pregnancy, and may alter the uterine environment at implantation which can impact embryonic survival (Ballarotti do Nascimento et al., 2013).

Table 2.4: Effect of condition score loss from calving to first service on conception rate to first service in high yielding dairy cows (Bourchier et al., 1987).

	Condition score loss			
	>1.5	1	0.5	>0
Conception rate	47	56	53	63

Furthermore, the cow commonly has substantial body reserves going into lactation, with farmers aiming to achieve a body condition score of around 3-3.5 (on a 1-5 scale) (Garnsworthy and Topps, 1982), which correlates with an average score of 5-5.5 on a 1-10 scale used in New Zealand (Roche et al., 2004). This level of body condition is aimed for in order to reduce dystocia, which would occur if the animals were too over-conditioned, and also allows the farmer to account for the inevitable loss of body reserves during early lactation (Garnsworthy and Topps, 1982; Kolver et al., 2002). However, the farmer must balance the condition of his cows, as higher body condition scores at calving are associated with a greater loss of body condition (Table 2.4) and, therefore, higher condition cows at calving tend to have a lower conception rate during early lactation due to this loss of condition (Garnsworthy and Topps, 1982; Bourchier et al., 1987). Additionally, it often takes these cows longer to regain the condition or body weight lost, and this may result in lower reproductive performance for a longer period of time postpartum, and may also have long-term effects on subsequent seasons (Garnsworthy and Topps, 1982; Veerkamp et al., 1994). Consequently, farmers need to ensure that cows are monitored regularly for live weight and body condition, and ensure management practices and supplementation levels are monitored during the season and managed to smooth over any fluctuations in feed availability that may occur during the season (Garnsworthy and Webb, 1999).

2.5 Thermo-neutral zone

Measures of biological energy are the foundation of most animal feeding systems, and heat energy produced from metabolism is the basis of thermal balance (Young, 1983). Therefore, energy is a common term used when describing the effects of the thermal environment on animals. Substrate energy, which is released through oxidation processes, appears as heat in the animal and is ultimately disposed to the environment through thermal exchange mechanisms (Young, 1983). Consequently, changes in environmental conditions or extreme climatic conditions have the ability to alter energy transfer between the animal and its environment (Gwazdauskas, 1985). Therefore, many of the effects of cold on animals are reflected by changes in appetite, digestive and metabolic functions (Young, 1983).

Optimum function of biological processes in dairy cows requires the cow to be within its thermoneutral zone (TNZ) (Broucek et al., 1991). The TNZ, by definition, is the zone in which an animal's heat production is independent of the ambient temperature (Young, 1983) and in dairy cows, this is generally between 6 and 15°C (Nardone et al., 2006). Figure 2.4 shows a schematic diagram depicting the TNZ in dairy cows. The zone within which the cow is comfortable is variable, and includes mildly warm and cool conditions. However, there is a point on each end of the scale at which the cow changes from being comfortable to being placed under stress.

The lower critical temperature (LCT) is the temperature below which the animal will need to increase its rate of metabolic heat production, in order to maintain homeothermy (Young, 1983). Below this temperature, metabolic heat production becomes increasingly dependent upon ambient temperature. However, a point of maximum heat production, known as the summit metabolism (Figure 2.5), is reached as a consequence of extreme cold, and continual exposure to even lower temperatures will result in the animal having a reduced capacity to produce metabolic heat, which leads to hypothermia, and eventually, death (Young, 1983). The LCT of an animal is not a constant number, and is dependent on various factors, including the rate of heat production in thermoneutral conditions, and the insulation provided by the coat and thermal tissues of the animal (Young, 1983).

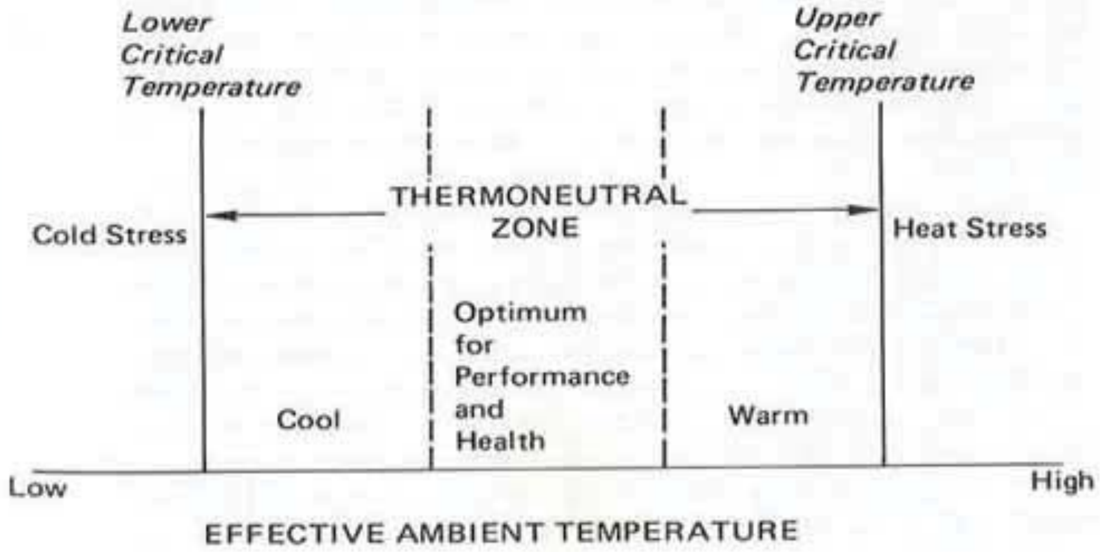


Figure 2.4: Schematic diagram depicting the thermoneutral zone, and associated terminology for dairy cows (Ames, 1987).

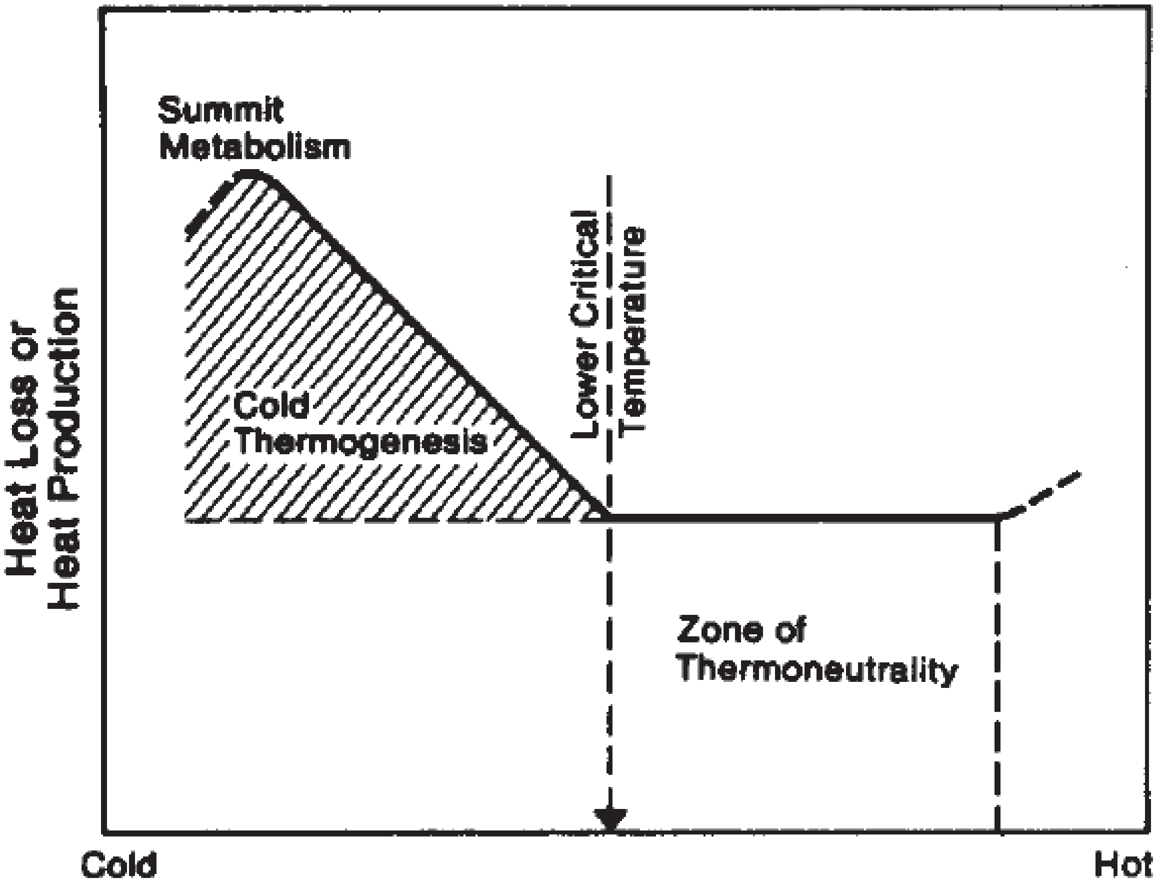


Figure 2.5: Relationship between the thermal environment and rate of heat loss or production of animals (Young, 1983).

The upper critical temperature (UCT) is the temperature above which the animal must increase the rate of respiration, in order to maintain thermoneutrality (Young, 1983). Again, there is a point of maximum respiration, above which body temperature increases as a result of the inability of the animal to dissipate the excess heat from the environment (Nienaber and Hahn, 2007). Above this temperature feed intake is reduced, in order to minimise heat produced, and as a consequence, production decreases (Bryant et al., 2007). The UCT is also not a constant figure and is mainly influenced by the condition of the animal, as fat cover will influence heat dissipation ability of the animal (Nienaber and Hahn, 2007). Other factors that can also contribute include genotype and respiratory diseases (Nienaber and Hahn, 2007).

In addition to the physical factors previously described, there are other factors that may influence both the LCT and UCT of an animal. Firstly, different breeds have been shown to differ in their abilities to withstand extreme temperatures, with Jersey cows being shown to have a higher threshold for heat than Friesian cows (Bryant et al., 2007), therefore the upper critical temperature would differ for these animals. Another large determinant of a cow's thermoneutrality is milk production level. A higher producing cow creates more heat, through metabolic processes, than a lower producing cow (Bryant et al., 2007). This additional heat will allow a higher producing cow to withstand cooler temperatures, in relation to a lower producing cow, however, it is also likely to be more susceptible to hotter temperatures, and production will be significantly affected (Bryant et al., 2007).

2.5.1 Effective ambient temperature

In Figure 2.4, the x-axis is labelled as effective ambient temperature (EAT), rather than just temperature, as the recorded temperature does not always reflect the actual conditions that cow may be exposed to. The effective ambient temperature is a term that is used to express the rate of energy transfer between the animal and its environment (Gwazdauskas, 1985). Factors that may affect the EAT include dry bulb temperature, relative humidity, solar radiation, wind speed and precipitation. For example, the dry bulb temperature may be 10°C, which generally lies within the thermoneutral zone (Nardone et al., 2006). However, if there is also a strong wind, and precipitation, the effective temperature is decreased, as the cow has a wet coat and is exposed to wind chill. Therefore, by using the effective ambient temperature, these factors are considered, and a more accurate temperature index is used to describe the effects of the environment.

2.6 Climatic stress as it affects reproduction

Many studies have been conducted in relation to heat stress, and its adverse effects on reproduction are extensively published. However, similar studies on cold stress and its effect on reproduction have not been widely researched (Gwazdauskas, 1985). A study carried out in Canada found lowest conception rates during February (winter), and highest in July (summer) (Mercier and Salisbury, 1947), suggesting that either day length or environmental temperatures were responsible for the variation in fertility. However, day length was shown to have no significant effect on the conception rate and fetal survival in Holstein heifers (Chebel et al., 2007). Subsequently, this would suggest that the major factor explaining the variation in fertility would therefore be the environmental temperature.

Environmental conditions that lead to a reduction in reproductive efficiency can be termed stress (Gwazdauskas, 1985). Evaluation of the adrenal function of cows during adverse weather conditions has received little attention; however, some studies have shown increased levels of plasma glucocorticoids (Gwazdauskas, 1985), particularly cortisol (Webster et al., 2008), when ruminants were exposed to cold environmental conditions. Increased levels of cortisol are an indication of a classic stress response which involves the activation of the hypothalamo-pituitary adrenal axis (Webster et al., 2008). The increase in cortisol may have been a result of the reduction in lying time observed during exposure to cold, wet conditions (Webster et al., 2008). In addition, when dairy cows are exposed to cold, wet and windy conditions, they cease grazing activity in order to seek shelter, shiver and/or orient themselves towards the wind and rain, in order to combat the effects of the environmental conditions, which is another indicator that these conditions cause discomfort and stress (Bryant et al., 2010). This physiological and behavioural effort, and the energy used to maintain body temperature occurs at the expense of production, growth, health and reproduction (Bryant et al., 2010).

Explanations for the reduction of fertility seen in cold environmental conditions are mostly speculation, but they include three major physiological responses to adverse weather conditions, evoked by the sympathetic nervous system: increased metabolic heat production, a higher cardiac output with a redistribution of blood flow, and a mobilisation of substrates for metabolism (Broucek et al., 1991). Increased metabolic heat production, and a number of other metabolic changes, including rate of digestion and absorption of nutrients are mediated by increased concentrations of thyroxine and tri-iodothyronine, which occur during adverse weather conditions (Gwazdauskas, 1985). These metabolic changes would suggest that during periods of adverse weather conditions, a higher intake would be beneficial, to allow for the increase in maintenance costs associated with maintaining body temperature, and the associated increase in cardiac output. However, during adverse weather conditions, decreases in feeding time have been observed, which would correspond to a reduction in intake (Webster et al., 2008). The decrease in intake would be likely to further impact on the animal's thermoregulatory ability (Gwazdauskas, 1985).

Previous studies have shown that increased, or *ad lib.* feed intakes of cows can cause lower circulating P4 concentrations, in relation to cows fed a restricted diet (Nolan et al., 1998). The increased feed intake results in a higher blood flow, in order to digest and utilise dietary energy, and this increased blood flow causes an increase in catabolism of P4, which causes the lower circulating concentration (Nolan et al., 1998). During periods of adverse weather conditions, cows increase cardiac output in order to increase heat production (Gwazdauskas, 1985). This increased cardiac output would cause a similar increase in blood flow through the animal, including increasing the rate of liver blood flow, commonly in conjunction with lower feed intakes (Bryant et al., 2007). Therefore, the increase in liver blood flow would also result in a decreased circulating concentration of P4 (Hafez, 1993). Lower circulating P4 concentrations are likely to cause a decrease in conception rate of cows which are inseminated during these periods, or an increase in embryonic mortality of cows that have recently been inseminated, as this hormone is critical in the establishment and maintenance of pregnancy (Ballarotti do Nascimento et al., 2013). However, depending on the stage of the oestrous cycle, this reduction in P4 may also cause a decline in behavioural oestrus, and consequently, lower submission rates (Ballarotti do Nascimento et al., 2013).

Energy and protein are the two major components of a cow's diet. It has long been recognised that cows have higher fertility during the mating period if they are gaining weight, compared to those cows losing weight (Ferraretto et al., 2014). This is due to the associated energy balance of the cows (Bilodeau-Goeseels and Kastelic, 2003). As dairy cows are bred to maintain a yearly calving interval, they tend to be in a negative energy balance (NEB) during the mating period. This negative energy balance occurs as the cow is unable to meet the sudden increase in nutrient demand placed by lactation from her current diet, and therefore must mobilise body reserves in order to fulfil these demands (Butler, 2000). As the cow mobilises these body reserves, she will lose body condition and this can have adverse effects on fertility. In addition, during cold, wet and windy conditions, cows have been shown to reduce feed intake, which would further enhance this loss of body condition (Bryant et al., 2007). Negative energy balance, or undernourishment in cows may result in an abnormal uterine environment, especially in relation to circulating hormone concentrations (Zavy, 1994; Butler, 2000). The physiological state of under-nutrition which occurs when the cow is in NEB suppresses LH secretion while also reducing ovarian responsiveness to LH, and prevents or delays ovulation (Butler, 2000). Furthermore, beef cows that have been fed a low energy diet whilst nursing their calves were observed to have lower levels of FSH and LH (Bilodeau-Goeseels and Kastelic, 2003), as a result of lower energy availability (Butler, 2000).

The energy balance of lactating dairy cows during the early lactation period has a regulatory role on the level of P4 increase during the first 2-3 ovulatory cycles (Butler, 2000). In cows with a negative energy balance, circulating P4 concentrations are reduced, which resulted in fewer cows expressing oestrus before the first post-partum ovulation, in relation to cows that had a more positive energy balance (Spicer et al., 1990). The mechanisms that facilitate the effect of energy balance on ovarian function are vague, but research has identified a possible effect of insulin-like growth factor-I (IGF-I) (Spicer et al., 1990).

In vitro studies have implicated IGF-I as a potent stimulator of bovine granulosa and luteal cell steroidogenesis (Spicer et al., 1990). This shows that IGF-I can stimulate production of hormones such as E2 from the ovarian granulosa follicles, and also P4 from luteal cells. Oestrogen levels peak just prior to the surge of LH that causes ovulation (Eilts and Paccamonti, 2004). This increase in E2 triggers the onset of oestrus, and stimulates behavioural signals such as mounting and increased activity (Bilodeau-Goeseels and Kastelic, 2003). Additionally, a high concentration of P4 prior to the release of E2 is necessary to sensitise the hypothalamus to E2 (Bilodeau-Goeseels and Kastelic, 2003). Consequently, the production of both P4 and E2 are important in the detection and submission of dairy cows during the breeding period. The concentration of IGF-I in the blood of cows is moderated by changes in nutrition, primarily variations in the protein and energy intake of the cows (Spicer et al., 1990). Therefore, the reduction in intake that occurs during adverse conditions may lead to a decrease in IGF-I, causing a decrease in production of both E2 and P4, which alters the hormonal balance during the oestrous cycle, resulting in a decrease in behavioural oestrus and submission rates and also decreasing conception rates (Bryant et al., 2007).

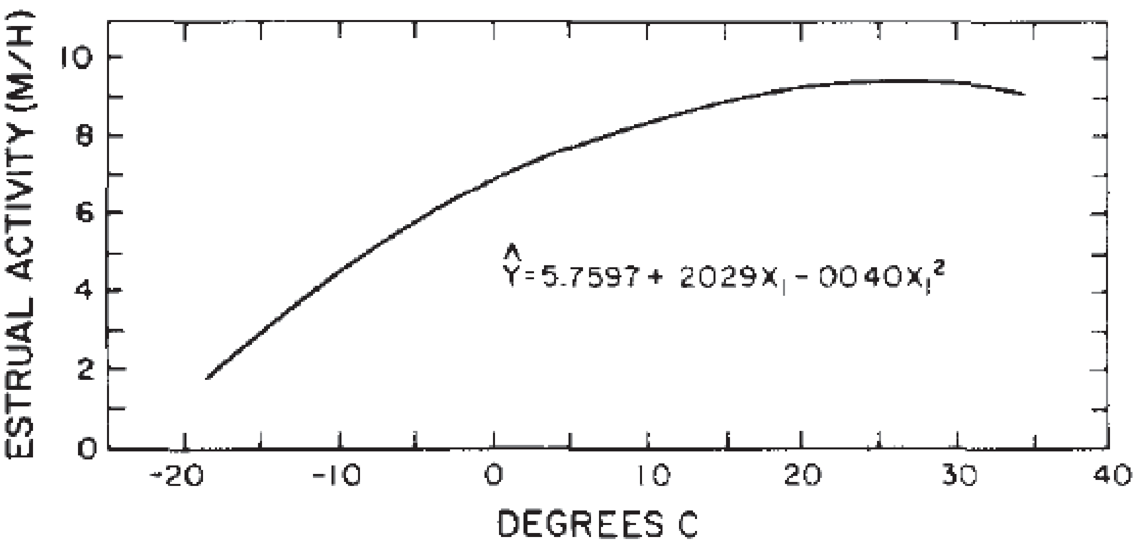


Figure 2.6: Relationship between the maximum daily temperature and oestrual activity (mounts/hour) (Gwazdauskas et al., 1983).

The decrease in E2 secretion is supported by behavioural studies carried out during adverse weather conditions which show a decrease in behavioural oestrus expression (Thatcher, 1974; Kilgour et al., 1977). In Figure 2.6, you can see that sexual activity, recorded as mounts per hour was significantly lower for temperatures below 0°C, for dairy cows observed in Australia (Gwazdauskas et al., 1983). Similarly, New Zealand beef cows which were exposed to wet, cold conditions displayed a decrease in sexual activities, such as mounting behaviour and activity, during the oestrus period (Kilgour et al., 1977). In a study carried out in the USA, maximum environmental temperature on the day of oestrus was used to assess the effects on oestrous activity (Thatcher, 1974). This study showed that the maximum environmental temperature was related to mounting activity in a curvilinear manner. Consequently, at temperatures below -10 °C, mounting activity was relatively low, and increased as temperature rose to 25 °C, before declining again above 30 °C (Figure 2.6) (Gwazdauskas et al., 1983). This would suggest that adverse weather conditions inhibit the expression of oestrus activity, and therefore, a decrease in submission rates would also arise. Consequently, farmers may benefit from increased observations or additional behavioural identification, other than mounting, in order to detect when cows are on heat during these periods.

2.7 Cold stress index

Due to the outdoor grazing systems used in the New Zealand dairy industry, dairy cows are regularly exposed to environmental conditions, including temperature, humidity, radiation, wind and rain (Bryant et al., 2010). The ability to provide an accurate description of the climate has been recognised as a difficulty in discussing the effect of weather on livestock (Ames, 1987). This difficulty is due to the fact that the cold conditions that affect performance of grazing dairy cows in New Zealand are not well defined (Bryant et al., 2007). In previous studies, climatic stress has been quantified using mechanistic and empirical models which account for the effects of different environmental conditions on the ambient temperature, such as temperature, radiation, and humidity (Mader et al., 2010). However, these models have been developed for use in Europe and North America, where the environmental conditions have less importance, due to the use of indoor dairy systems (Bryant et al., 2010).

Due to this lack of data for outdoor grazing systems, a number of studies were carried out to observe behavioural responses to both hot and cool conditions (Tucker et al., 2008; Schutz et al., 2010). These studies offered cows the option of shade, and recorded the conditions during which the cow would choose shade, rather than being exposed to the conditions (Tucker et al., 2008). The results of these New Zealand studies were used to develop and validate both a heat and cold stress index model for use in the New Zealand climate (Bryant et al., 2010).

2.9 Conclusion

Farmers regularly blame the weather for low submission and conception rates to artificial insemination (AI) (Burke, 2014). This is because cows that appear to be cycling well, prior to the planned start of mating (PSM), then appear to stop cycling during the mating season, commonly in conjunction with adverse weather conditions, such as exposure to short or extended periods of cold, wet and windy conditions (Burke, 2014). Reviewing current literature, we can determine that when cows are placed outside their comfort zone, they are exposed to stress and this affects all aspects of production, including milk production, growth and reproduction. Although there is evidence that cold conditions do have effects on the reproductive function of dairy cows (Kilgour et al., 1977; Pennington et al., 1985), it is not well understood. In addition, the cold conditions that affect performance of grazing dairy cows are not well defined (Bryant et al., 2007). Having reviewed the available literature, it appears there is a relationship between adverse weather events and submission and conception rates, so the second objective of this study was to obtain herd data and analyse the effects of climate on submission and conception rates in order to support or refute the literature. The results from this analysis may then be used to provide information on the mechanisms that may cause this relationship so that farmers can reduce the effects of adverse weather events.

Chapter 3 : Materials and Methods

3.1 Herd data

Mating records (including submission dates, pregnancy test results, and calving dates), lactation records and pedigree information were provided by CRV Ambreed for the 2013 and 2014 seasons. The daily submission rates of these herd were initially analysed to identify any herds that had used synchronisation techniques on these cows. Synchronies were identifiable by a significant increase in submission rates for 1-3 consecutive days. These herds were excluded from the dataset, as synchronisations often used timed AI, where animals are not observed for oestrus, but inseminated a set amount of time after hormonal treatment, and therefore, would skew the results. After excluding these herds, there were 20 herd remaining for analysis. These herds were located in 5 different regions within New Zealand, with 1 region in the South Island (Central Otago, 2 herds), and 4 regions in the North Island (Manawatu (1), Waikato (12), Auckland (2), Northland (3)).The raw data was filtered for the following parameters. Animals were first sorted by herd and cow ID, and any animals that did not have mating records for the 2013 mating period were removed as this is the key parameter that is being analysed. Following this, any cows with no calving date in 2013 were also excluded, as these records were required to calculate days in milk (DIM) and predicted milk yield on the day of submission. The herd records used had not recorded natural matings, and therefore, the following analysis is restricted to the artificial breeding period of these herds. After the data had been filtered, the dataset contained a total of 6664 cow mating records from 20 herds.

For herds that did not have pregnancy test records (7 herds), date of conception was calculated based on the last submission date of each cow. These dates were also checked against the calving dates for 2014, and any cows that did not calve in 2014 were assumed to be empty, and were not allocated a pregnancy date. Using the submission and pregnancy dates, the days from the start of breeding until first service (SBFS) and the start of breeding until conception (SBCO) were calculated and then used to determine the 21 and 42 day submission rates and 21-day and 6-week in-calf rates of each cow.

$$\text{SBFS} = \text{ date of first mating} - \text{start of breeding date}$$

$$\text{SBCO} = \text{date of conception} - \text{start of breeding date}$$

Submission and in-calf rates are typically expressed as a percent of the herd submitted or conceived within a set time period (Bilodeau-Goeseels and Kastelic, 2003). For this analysis, submission rates are expressed as binomial terms for each individual cow; therefore, they were recorded as either being submitted (1) or not submitted (0) within each time period (21 or 42 days). When analysed on a whole herd or whole data set basis, this would calculate an average, resulting in the percentage of cows submitted during each time period. A 21 day period has been selected as this represents the average length of a cow's oestrous cycle, with a typical range from 18-24 days (Bilodeau-Goeseels and Kastelic, 2003). Therefore using 21 and 42 days allows for a representation of the first two cycles in which the cows should be eligible to be bred. In-calf rates and conception to each service were also analysed in this manner, using SBCO to determine whether or not the cow conceived within each specified time period, and therefore, whether or not the cow conceived to a specific service.

The breed of each animal was provided in genotype format (eg FFJF) (Rosati et al., 2007). These genotypes were then used to calculate the breed 16ths of the cow. Anything F14 or above was classed as Friesian, anything J14 and above was classed as Jersey, and any cows that fell between these were classed as crossbred (DairyNZ and LIC, 2014).

Herd test results from each cow were used to model lactation curves for the season. Herd tests are a common measuring tool in New Zealand, with recommendations for around three to four tests per lactation (DairyNZ, 2014). The test involves collecting individual milk samples from each cow in the herd for two consecutive milkings (Back and Lopez-Villalobos, 2007). Milk yield was recorded on the day of collection, and milk samples were analysed for composition of fat and protein as well as somatic cell count on a combined infrared milk analyser and cell counting machinery (Combi-Foss [FT+200 and FT+400], Foss Electric, Hillerød, Denmark) at CRV Ambreed (Hamilton, New Zealand). The milk yield from each herd test was used to calculate a predicted lactation curve for each cow, using legendre polynomials (Munera Bedoya et al., 2014). The lactation curve was then used to determine the predicted milk yield for each day that the cow was submitted for mating.

3.2 Meteorological data

Meteorological data was obtained using the National Institute of Water and Atmospheric Research (NIWA) CliFlo database. Map co-ordinates were supplied for each herd by CRV Ambreed and using these co-ordinates, the nearest climate station was selected in order to obtain the most accurate data for each herd. The nearest climate station was determined from a program which used the following formula to calculate the station with the minimal distance from the farm (Levy, 2014).

$$\text{Distance} = \sqrt{[(\text{station longitude} - \text{farm logitude})^2 - (\text{station latitiude} - \text{farm latitude})^2]}$$

After selecting the closest station, the data obtained included daily measures of maximum and minimum temperatures (°C), rainfall (mm) and wind speed (m/s).

3.2.1 Cold Stress Index

In order to define the conditions which the cows are exposed to in this study, a cold stress index (CSI) was calculated using the following formula (Donnelly, 1984):

$$\mathrm{CSI} = [11.7 + (3.1 \times \mathrm{WS}^{0.5})] \times (40 - \mathrm{T}) + 481 + \mathrm{R}$$

Where WS is the mean daily wind speed (m s^{-1}), T is the mean daily temperature (°C) calculated as the average of the maximum and minimum temperature, and R= 418(1-$e^{-0.04rain}$) where rain is the total daily rainfall in millimeters (Donnelly, 1984). The use of this formula allows both wind speed and rainfall to be taken into account, which are both key determinants of the effective temperature that grazing cows are exposed to.

Furthermore, this data was also used to calculate a 3-day average CSI (the day of submission and the 2 days prior). It has been shown that the 2 days prior to the test date had the greatest influence on milk yield in dairy cows (West et al., 2003). Furthermore, a significant association was found between the 3-day CSI and milk yield and fat/protein concentrations in three different breeds of dairy cows (Bryant et al., 2007). Due to these effects, a three-day average CSI was calculated to determine the differences between three-day and single-day CSI.

3.3 Statistical Analysis

Analyses of variance were performed with the statistical package SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) using the MIXED procedure for continuous variables (e.g. predicted milk yield at mating) and the GLIMMIX procedure for binomial variables (e.g. submitted or not submitted for mating). Two linear models were explored:

Model 1:

$$y_{ijkl} = \mu + H_i + B_j + L_k + C_l + BL_{jk} + \beta_1 c_t + e_{ijkl}$$

Model 2:

$$y_{ijkl} = \mu + H_i + B_j + L_k + C_l + BL_{jk} + \beta_2 p_t + \beta_3 m_t + \beta_4 n_t + \beta_5 r_t + \beta_6 w_t + e_{ijkl}$$

where y_{ijkl} is the dependent variable. For this research the dependent variables include 21 and 42 day submission rates (SR_{21}, SR_{42}) 21 day and 6-week in-calf rates (ICR_{21}, ICR_{42}) pregnancy to the first or second service (PG_FS or PG_SS) and predicted milk yield on the day of service (PMY). μ is the population mean, H_i is the fixed effect of herd i, B_j is the fixed effect of breed j (j = Friesian, Jersey and Crossbred), L_k is the fixed effect of lactation number k (k= 2, 3, 4, 5, 6+), C_l is the fixed effect of calving month l (l= June-October), BL_{jk} is the fixed effect of the interaction between breed j and lactation k, c_t is the cold index at day t, β_1 the regression coefficient that measures the effect of cold index at day t on the response variable, p_t is the predicted milk yield at day t, β_2 the regression coefficient that measures the effect of predicted milk yield at day t on the response variable, m_t is the maximum temperature at day t, β_3 the regression coefficient that measures the effect of maximum temperature at day t on the response variable, n_t is the minimum temperature at day t, β_4 the regression coefficient that measures the effect of minimum temperature at day t on the response variable, r_t is the rainfall in mm at day t, β_5 the regression coefficient that measures the effect of rainfall at day t on the response variable, w_t is the wind speed in ms^{-1} at day t, β_6 the regression coefficient that measures the effect of wind speed at day t on the response variable and e_{ijkl} is the residual error associated to the observation y_{ijkl}.

In order to gain a more in depth understanding of the effects of cold stress on the submission and conception rates of the cows analysed in this project, the cold stress values were split into clusters using the PROC FASTCLUS procedure in SAS (SAS, 2013), which groups cows together based on the level of cold stress they were exposed to on the date of first submission. For this data, the cold stress values were split into four different groups, with the level of cold stress increasing with each subsequent group. Statistical analyses were carried out on these four clusters to first show differences in the effect of the key climatic variables used to calculate the cold stress level and also the effects of different levels of cold stress on submission and conception rates.

3.4 Data cleaning

No research on cold stress using New Zealand herd data has previously been published, and part of the learning for this project was how to clean the data for this process. The procedure used in the current study is outlined below, so that future work can build on this to develop a streamlined system.

The data that was used for this study was raw herd data, as opposed to data recorded by researchers. Therefore, as farmers have their own preferences for recording, there was a tendency for data to be missing, or for different farms to record different parameters and the data had to be edited in order to make it suitable for analysis. In order to make the raw data into a format that was suitable for analysis, each herd was first sorted by cow identification number, and any cows that did not have mating records for the 2013 mating season were removed. These cows were identifiable as cows with no recorded mating submission dates, and were removed as submission rate was the key parameter being analysed in this study. During this stage, it was also important to ensure that all data for each cow was in a single row, and therefore, submission dates were rearranged so that they were in sequential columns rather than rows, which is the standard output. Secondly, cows were also removed if they did not have a calving date for 2013. This was important as the 2013 calving date was used to calculate days in milk (DIM) and predicted milk yield (PMY) at mating. Therefore, no first mating heifers were used in this analysis.

In-calf rates and likelihood of pregnancy was predicted based on pregnancy testing records, however not all herds pregnancy tested and for these herds, date of conception was calculated based on the last submission date of each cow. These dates were also checked against the calving dates for 2014, and any cows that did not calve in 2014 were assumed to be empty, and were not allocated a pregnancy date. Using the submission and pregnancy dates, the days from the start of breeding until first service (SBFS) and the start of breeding until conception (SBCO) were calculated and then used to determine the 21 and 42 day submission and in-calf rates of each cow. Start of breeding for each herd was determined by the earliest submission date that was followed with submissions on at least 4 out of 7 consecutive days (Creagh et al., 2013). If herds had lone submissions significantly outside this mating period, these submissions were considered outliers and were removed from the data.

As discussed earlier, these reproductive variables were expressed in binomial terms, as either being submitted or having conceived (1) or not submitted or conceived (0) within each time period (21 or 42 days). In order to create these variables, the IF statement function in excel were used, calculated from the SBFS or SBCO parameters, with the IF statement determining whether the parameter was above or below 21 or 42 days for each parameter respectively. Pregnancy to first and second service were also expressed as binomial terms and calculated using the IF statement. A column was created which calculated the difference (in days) between pregnancy date and first or second submission date. The IF statement was then used, with a positive pregnancy occurring when the days between the two variables was zero, any other number was seen as not conceiving to that submission.

Milk production plays a large role in the energy allocation of the cow, and has a negative correlation with reproductive ability of the cows. Therefore, milk yield on the day of submission was also included in the analyses, in order to account for any differences in production levels between cows. In order to determine milk yield, results from herd tests throughout the season were used. Herd test data was exported into excel, and had to be exported one herd test at a time. The results from each cow were copied into the data set in order of date of test. This parameter varied largely between herds, with 2 herds having no recorded herd test results, and the remaining 18 herds varying between 1 and 5 herd tests during the season. All results available for each cow were included into the data set, and then used to model lactation curves for the season which was calculated in SAS using legendre polynomials (SAS, 2013), and was subsequently used to predict the milk yield on the day of submission. It is interesting to note that there was actually a slightly positive correlation between PMY and SR21 and SR42, indicating an increase in submission rate with a higher PMY (Table 4.3). However, this trend was not continued with other reproductive parameters, with a negative correlation between PMY and 21d-ICR, 6wk-ICR, PFS and PSS.

For the climatic data, a program was run, as earlier, which sourced data from the nearest climate station for each herd. Each parameter was expressed in the correct form for analysis. The only changes made to this data set were to use these parameters to calculate the cold stress index, as described earlier. This data was then imported into SAS and paired with the reproductive data set to create a master set where the climatic data from the cow’s herd was added to the data row for that cow from the specified submission date.

Further studies in this area will need to be large-scale, using herd data from multiple seasons. Therefore the factors detailed above would need to be considered so that the data process can be streamlined, as it is time-consuming and labour-intensive.

Chapter 4 : Results

4.1 Descriptive statistics

The number of animals in each breed, lactation and calving group, as well as the variables analysed for this project are presented in Tables 4.1 and 4.2 respectively. These statistics represent the average of all 6664 cows from the 20 herds included in this study, which contain a mixture of three key New Zealand dairy breeds, Friesian (60%), Jersey (11%) and Friesian x Jersey crossbred (29%). The mean lactation number was 4 with a standard deviation of 1.5. The mean calving date was 08 August, with a standard deviation of 23 days.

4.1.1 Herd statistics

As seen in Table 4.2, the average number of days from start of breeding until first service (SBFS) was 14 days with a standard deviation of 11 days. The average number of days from start of breeding until conception (SBCO) was 21 days, with a standard deviation of 15 days. The mean 21-day submission rate (SR21) was 77% with a standard deviation of 41% and the mean 42-day submission rate (SR42) was 97% with a standard deviation of 16%. The mean 21-day in-calf rate (21d-ICR) was 45% with a standard deviation of 49% and the mean 6-week in-calf rate (6wk-ICR) was 71% with a standard deviation of 45%. The mean pregnancy rate to first service (PFS) was 54% with a standard deviation of 49% and the mean pregnancy to second service (PSS) was 69% with a standard deviation of 46%. The mean predicted milk yield at first service (PMYFS) was 25.1L with a standard deviation of 6.2L and the mean predicted milk yield at second service (PMYSS) was 24.8L with a standard deviation of 6.3L.

4.1.2 Meteorological Statistics

Meteorological data was obtained for June until December 2013 from stations which closely represented the climates on each farm, with 18 herds located in the North Island, and 2 located in the South Island (Table 4.2). The mean maximum temperature was 17°C with a standard deviation of 4°C and the average minimum temperature was 8°C with a standard deviation of 4°C. The mean wind speed was 2.8m/s with a standard deviation of 1.5m/s. The average daily rainfall was 2.9mm with a standard deviation of 7.1mm and the mean cold stress index was 967 $kJm^{-2}h^{-1}$ with a standard deviation of 101 $kJm^{-2}h^{-1}$.

Table 4.1: Number of cows in each breed, lactation and calving month group for the analysis of the effects of cold stress on the fertility of seasonal breeding dairy cows in New Zealand.

Variable	Category	Number of cows
Breed	Friesian	3777
	Jersey	732
	Crossbred	1806
Lactation	≤2	1512
	3	972
	4	1084
	5	943
	6+	1770
Calving month	June	217
	July	1967
	August	2866
	September	862
	October	161

Table 4.2: Number of records (N), mean, standard deviation (SD), minimum and maximum values of the key characteristic traits used for the analysis of the effects of cold stress on the fertility of seasonal breeding dairy cows in New Zealand.

Trait[a]	N	Mean	SD	Min	Max
Lactation	6283	4.08	1.55	2	4
Mean calving date (days)	6073	08-08	23	04-06	25-10
SBFS (days)	6317	14.70	11.32	0	90
SBCO (days)	4989	21.53	15.49	0	96
SR21	6317	0.77	0.42	0	1
SR42	6317	0.97	0.17	0	1
21d-ICR	6317	0.45	0.50	0	1
6wk-ICR	6317	0.72	0.45	0	1
PFS	6317	0.55	0.50	0	1
PSS	2975	0.69	0.46	0	1
PMYFS (L)	5063	25.10	6.22	6.5	53.2
PMYSS (L)	1831	24.83	6.26	7.3	49.7
Max temp (°C)	10300	17.94	4.26	3.7	30.5
Min temp (°C)	10300	8.17	4.35	-3.8	19.4
Wind (ms^{-1})	10300	2.82	1.45	0	10.4
Rain (mm)	10300	2.98	7.09	0	77.8
Cold index	10300	967.46	101.94	795.27	1312.99

[a] SBFS: the number of days from the start of breeding until first service, SBCO: the number of days from the start of breeding until conception, SR21: 21-day submission rate, SR42: 42-day submission rate, 21d-ICR: 21-day in-calf rate, 6wk-ICR: 6-week in-calf rate, PFS: pregnancy to first service, PSS: pregnancy to second service, PMYFS: predicted milk yield on the day of first service and PMYSS: predicted milk yield on the day of second service.

4.2 Reproductive statistics

Figure 4.1 shows the distribution of first mating dates for all 6664 cows analysed, which reveals a relatively normal distribution, concentrated over the October-November 2013 period. This Figure is also supplemented by Figure 4.2 which shows the days from the start of breeding until first service for each cow analysed. It is important to note that Figure 4.2 takes the date of first submission and accounts for the differences in start of breeding date between herds and shows that 77.26% of cows were submitted within the first 21 days from the start of the breeding period of their herd.

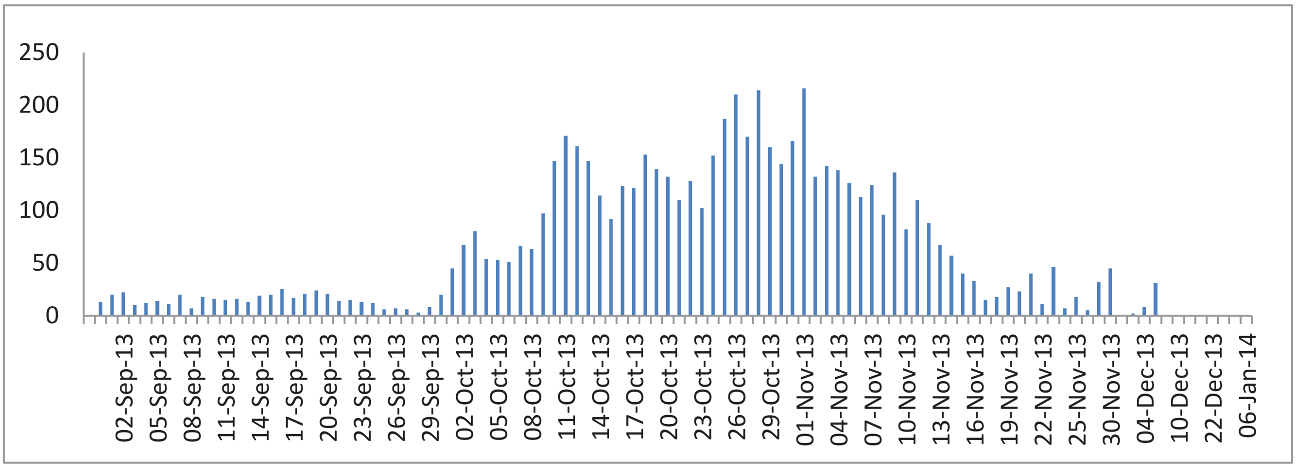


Figure 4.1: First submission date of each cow for the 2013 mating period recorded for the analysis of the effects of cold stress on the fertility of seasonal breeding dairy cows in New Zealand.

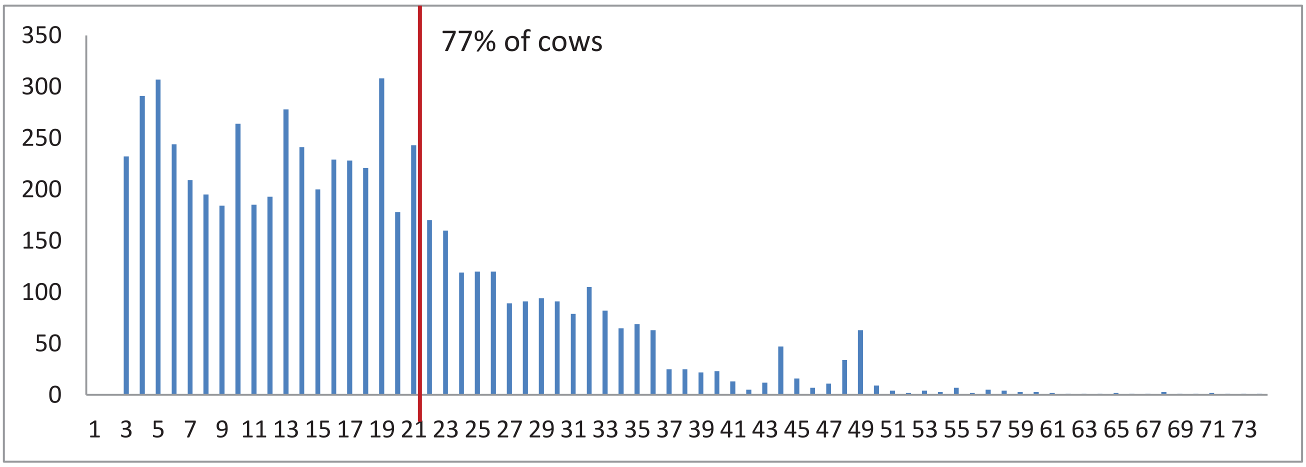


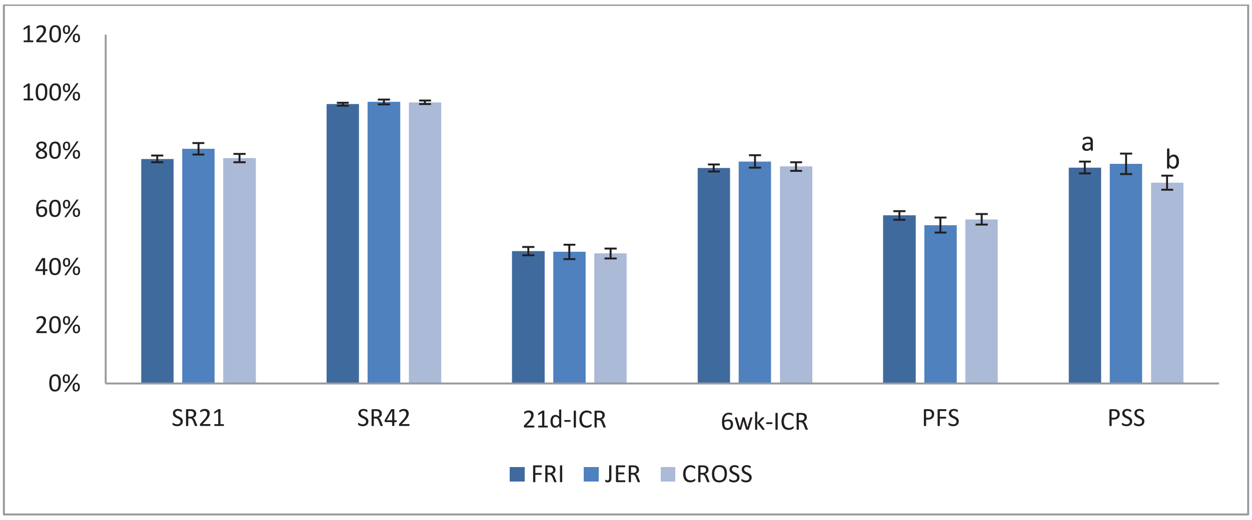
Figure 4.2: Days from start of breeding until first service for each cow in the analysis of the effects of cold stress on the fertility of seasonal breeding dairy cows in New Zealand.

4.2.1 Effects of breed, lactation and calving month

No differences were found between breeds for submission rate, in-calf rate and pregnancy to first and second service except between Friesian and Crossbred cows for PSS, with Friesian cows having a 5% higher rate of pregnancy than Crossbred cows (P=0.0193) (Figure 4.3).

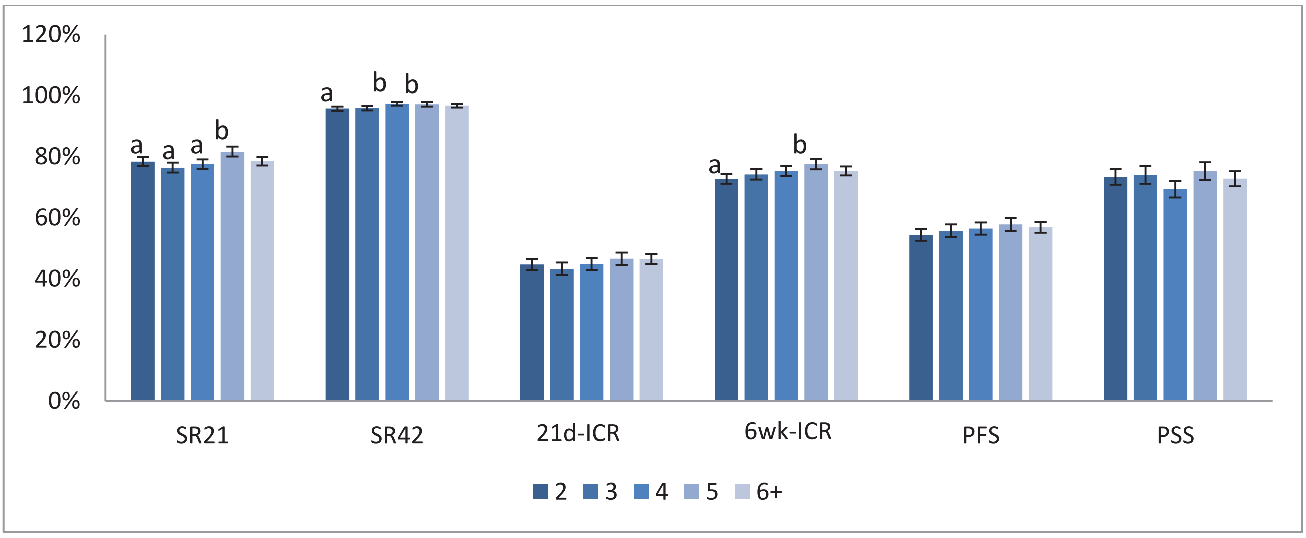
There were differences in submission rates at 21 and 42 days and 6-week in-calf rates for lactation number (P<0.005), with a trend towards increased submission and conception rates in older animals (Figure 4.4).

Figure 4.5 shows there are consistent differences (P<0.05) between all months of calving for all variables with the exception of PSS. The trend is for reproductive performance to decrease the later the cow calves within the season. For 42-day submission rate (SR42) and 6-week in-calf rates (6wk-ICR), there were no differences between cows calving in June and July (early calvers) (P>0.05) as well as no differences in late calving cows (September-October) for 6wk-ICR (P>0.05).



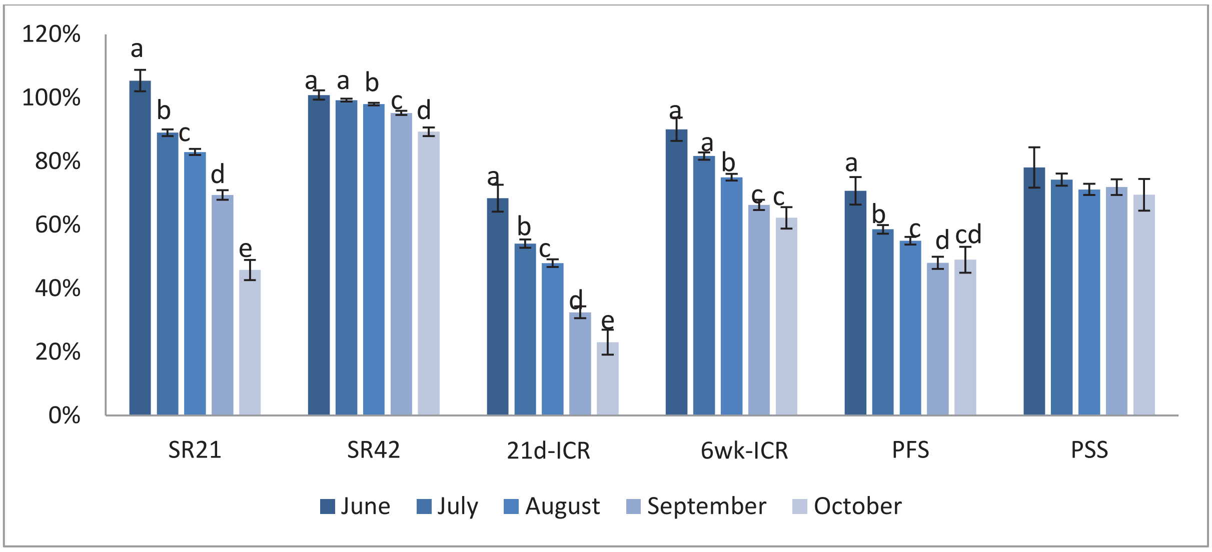
Where SR21 and SR42 are 21- and 42- day submission rates, 21d-ICR and 6wk-ICR are 21-day and 6-week in-calf rates, PFS is pregnancy to first service and PSS is pregnancy to second service.

Figure 4.3: Least square means of fertility traits for Holstein-Friesian (F), Jersey (J) and first cross F × J cows in New Zealand dairy cows for the 2013 breeding season. Different letters (a, b) indicate a significant (P<0.05) difference between bars.



Where SR21 and SR42 are 21- and 42- day submission rates, 21d-ICR and 6wk-ICR are 21-day and 6-week in-calf rates, PFS is pregnancy to first service and PSS is pregnancy to second service.

Figure 4.4: Lactation differences for key reproductive traits for dairy cows in New Zealand during the 2013 breeding season. Different letters (a, b) indicate a significant (P<0.05) difference between bars.



Where SR21 and SR42 are 21- and 42- day submission rates, 21d-ICR and 6wk-ICR are 21-day and 6-week in-calf rates, PFS is pregnancy to first service and PSS is pregnancy to second service.

Figure 4.5: Calving month differences for key reproductive traits for dairy cows in New Zealand during the 2013 breeding season. Different letters (a, b, c…) indicate a significant (P<0.05) difference between bars.

4.3 Meteorological Statistics

Regression coefficients of the key reproductive parameters are displayed in Table 4.3. The negative association between maximum temperature and submission and in-calf rates shows that as the maximum temperature increases, both the submission and in-calf rates decrease, whereas the likelihood of pregnancy to first and second service had an opposite, positive relationship.

The variation between positive and negative results for minimum temperature shows that there was no consistent effect of this variable.

Wind speed had a positive and significant relationship with 21-day submission rate and 21-day and 6-week in-calf rates, showing that an increase in wind speed led to an increase in the 21-day submission rate and 21-day and 6-week in-calf rates.

The cold stress index also had a positive relationship with the 21- and 42-day submission rates, indicating an increase in these variables as the cold stress levels increased. However, the relationships with in-calf rates and likelihood of pregnancy were negative, indicating a decrease in these traits as the cold stress levels increased.

Predicted milk yield had a positive relationship with the submission rate of the cows, indicating an increase in submission rate as milk yield increased, but a negative relationship with the in-calf rate, and with the likelihood of pregnancy which was significant (P=0.0002) for the first service.

Table 4.4 shows the relationships between predicted milk yield and cold stress index on these key reproductive parameters on the day of first submission. Predicted milk yield had a slightly positive and significant correlation with the 21- and 42-day submission rates (P=0.0006), whilst having a slightly negative and non-significant correlation on the 21-day and 6-week in-calf rates (P=0.08), indicating an increase in submission rate as a result of increased milk yield but also causing a decrease in in-calf rate. The cold stress index (CSI) had a slightly positive relationship with all parameters (P values ranged from <.0001 – 0.1654), indicating an increase in cold stress causes an increase in submission, in-calf and pregnancy rates.

Table 4.5 shows the relationships between individual meteorological effects, accounting for the predicted milk yield on the day of first submission. Maximum temperature had a negative correlation with both the 21- and 42-day submission and in-calf rates, indicating an increase in maximum temperature caused a decrease in submission and in-calf rates. In contrast, maximum temperature had a positive correlation with PFS and PSS, indicating an increase in these variables as the temperature increased.

Minimum temperature had a positive relationship with the 21-day submission rate and a negative relationship with the 42-day submission rate as well as the 21-day and 6-week in-calf rates, indicating an increase in minimum temperature caused an increase in 21-day submission rate, but a decrease in in-calf rates and 42-day submission rate.

Rainfall and wind speed had a slightly positive correlation with 21-day submission rate and 21-day and 6-week in-calf rates, indicating an increase in submission and in-calf rates during period of increased rain and wind.

Table 4.3: Regression coefficients for submission rates, in-calf rates and pregnancy to first and second service on climatic variable and milk yield for New Zealand dairy cows during the 2013 breeding season, excluding effects of herd, breed, lactation and calving month.

Covariable	SR21[a]		SR24[b]		21-dICR[c]		6wk-ICR[d]		PFS[e]		PSS[f]	
	Estimate	P Value	Estimate	P Value	Estimate	P Value	Estimate	P Value	Estimate	P Value	Estimate	P Value
Max Temp	-0.01356	<.0001	-0.01623	<.0001	-0.00558	0.0717	-0.0051	0.0007	0.01455	<.0001	0.001595	0.6542
Min Temp	-0.1268	<.0001	0.01708	0.0001	-0.08003	<.0001	-0.2182	<.0001	-0.03183	0.0035	0.04660	0.0272
Rainfall	-0.00225	0.4130	-0.00062	0.6890	0.000294	0.8337	0.0015	0.4431	0.00049	0.7688	0.002225	0.3634
Wind Speed	0.08558	<.0001	-0.00347	0.1995	0.04841	<.0001	0.0250	<.0001	-0.00637	0.3128	-0.01867	0.0998
CSI[g]	0.001406	<.0001	0.000181	<.0001	0.000851	<.0001	0.0004	<.0001	-0.00012	0.1654	-0.00025	0.0407
PMY[h]	0.004395	0.0006	0.002209	0.0004	-0.00301	0.0823	-0.0026	0.0883	-0.00657	0.0002	-0.00307	0.2476

[a] SR21: 21-day submission rate.
[b] SR42 : 42-day submission rate.
[c] 21d-ICR: 21-day in-calf rate.
[d] 6wk-ICR: 6-week in-calf rate.
[e] PFS: pregnancy to first service.
[f] PSS: pregnancy to second service.
[g] CSI: cold stress index.
[h] PMY: predicted milk yield on the day of mating.

Table 4.4: Regression coefficients of predicted milk yield (PMY) and cold stress index (CSI) on reproductive characteristics accounting for the fixed effects of herd, breed, lactation and calving month.

Trait[a]	PMY[b]			CSI[c]		
	Estimate	Standard Error	P Value	Estimate	Standard Error	P Value
SR21	0.005210	0.001389	0.0001	0.001549	0.000072	<.0001
SR42	0.002238	0.000635	<.0001	0.000199	0.00003	<.0001
21d-ICR	-0.002480	0.001720	0.1575	0.000923	0.000089	<.0001
6wk-ICR	-0.002360	0.001547	0.1298	0.000564	0.000800	<.0001
PFS	-0.006310	0.001794	0.0004	0.000110	0.000093	0.2106
PSS	0.003080	0.002680	0.2481	0.000360	0.000145	0.0128

[a] SR21: 21-day submission rate, SR42: 42-day submission rate, 21d-ICR: 21-day in-calf rates, 6wk-ICR: 6-week in-calf rates PFS: pregnancy to first service and PSS: pregnancy to second service.

[b] PMY: the predicted milk yield on the day of mating

[c] CSI: cold stress index..

Table 4.5: Regression coefficients for individual climatic events on reproductive characteristics accounting for the fixed effects of herd, breed, lactation and calving month and the variable effect of predicted milk yield.

Trait[a]	Max temp			Min temp			Rainfall			Wind speed		
	Estimate	Standard Error	P Value	Estimate	Standard Error	P Value	Estimate	Standard Error	P Value	Estimate	Standard Error	P Value
SR21	-0.04124	0.001401	<.0001	0.04647	0.002015	<.0001	0.005170	0.001270	<.0001	0.05701	0.004314	<.0001
SR42	-0.01730	0.000962	<.0001	-0.01152	0.000915	<.0001	-0.00098	0.000558	0.0526	0.00273	0.001927	0.1075
21d-ICR	-0.02270	0.002690	<.0001	-0.01621	0.002204	<.0001	0.004292	0.001522	0.0056	0.02950	0.005241	<.0001
6wk-ICR	-0.01434	0.002413	<.0001	-0.01621	0.002256	<.0001	0.003274	0.001361	0.0124	0.01494	0.004697	<.0001
PFS	0.01052	0.002793	0.0002	-0.00418	0.002618	0.1086	0.000246	0.001572	0.8791	-0.00380	0.005432	0.5312
PSS	0.005114	0.003959	0.1522	0.000424	0.004059	0.9529	-0.00380	0.002029	0.0732	-0.02378	0.009592	0.0161

[a] SR21: 21-day submission rate, SR42 :42-day submission rate, 21d-ICR: 21-day in-calf rates, 6wk-ICR: 6-week in-calf rates , PFS: pregnancy to first service and PSS: pregnancy to second service.

The relationship between temperature, wind, rain and predicted milk yield on the date of first submission are similar between those cows submitted for first insemination within the first 21-day period of breeding, and those submitted after the first 21 days. The one variable that differs is rainfall, which has a negative relationship with cows submitted within the first 21 days and a positive effect for those cows not submitted within the first 21 days (Tables 4.6 and 4.7).

The first submission date is showing the submission rate of cows for their first submission during the mating period. Table 4.6 shows that there is a positive correlation between maximum temperature and the date of first submission, indicating an increase in submission rate for first insemination as the maximum temperature increases, if the first insemination occurred within the first 21 days of mating. Wind speed has a large negative correlation on the date of first submission, indicating a decrease in the number of cows mated as wind speed increases during the first 21 days. Minimum temperature has a negative relationship with first submission date, indicating a decrease in submission rates for first insemination as minimum temperature increases, and this effect increases the later that cows are submitted (Table 4.7).

The relationships of these same variables on the likelihood of pregnancy to first service are more varied between cows submitted early or late (Tables 4.6 and 4.7). However, in contrast to the first submission date, the effects on pregnancy to first service have large P-values (>0.05) indicating they are not significant effects. Maximum temperature shows a slight positive relationship with pregnancy to first service for cows submitted within the first 21 days, indicating an increase in pregnancy rates as the maximum temperature increases; however, this increase is much smaller than that seen for first submission date (Table 4.6). Furthermore, predicted milk yield on day of first submission has a negative relationship with pregnancy to first service, indicating that as milk yield increases, likelihood of becoming pregnant decreases (Table 4.6).

For cows submitted for first insemination after the first 21 days of breeding, there is a positive relationship between wind speed and pregnancy to first service, indicating an increase in the likelihood of pregnancy to first service as wind speed increases (Table 4.7). All other factors have no effect on the likelihood of becoming pregnant to first service.

Table 4.6: Regression coefficients for first submission date and pregnancy to first service on climatic variables and predicted milk yield for cows that were submitted for first service in the first 21 days of mating. Bold numbers indicate significant variables.

	First submission date		PFS[c]	
Covariable	Estimate	P	Estimate	P
Max temp	**0.6450**	<0.0001	**0.009519**	0.0439
Min temp	-0.2230	0.0867	-0.00202	0.8734
Rainfall	**-0.1250**	<0.0001	0.000794	0.6620
Wind speed	**-1.0736**	<0.0001	-0.00386	0.6002
CSI[a]	-0.02211	<0.0001	-0.00013	0.1911
PMY[b]	**-0.06077**	0.0041	**-0.00823**	<0.0001

[a] PFS: pregnancy to first service.

[b] CSI: cold stress index.

[c] PMY is predicted milk yield on the day of service.

Table 4.7: Regression coefficients for first submission date and pregnancy to first service on climatic variables and predicted milk yield for cows that were submitted for first service after the first 21 days of mating. Bold numbers indicate significant variables.

Covariable	First submission date		PFS[c]	
	Estimate	P	Estimate	P
Max temp	**0.9633**	<0.0001	-0.00419	0.3295
Min temp	-1.8210	0.0155	0.08263	0.0746
Rainfall	**0.01831**	0.7956	-0.00389	0.3715
Wind speed	**-0.8656**	0.0001	**0.04421**	0.0015
CSI[a]	-0.02662	<0.0001	0.000204	0.3313
PMY[b]	-0.08194	0.1448	-0.00286	0.4093

[a] PFS: pregnancy to first service.

[b] CSI: cold stress index.

[c] PMY is predicted milk yield on the day of service.

4.4 Cold stress index

The mean CSI is very similar for both the single and three-day average values calculated, with the three-day average having a smaller range than the single day CSI (Table 4.8). Due to the similarities between the two indices, only the single-day CSI will be used for further analyses.

Table 4.8: Table comparing the mean, standard deviation, minimum and maximum values for the cold stress index and three-day cold stress index ($kJm^{-2}h^{-1}$). Figures are indicative of the New Zealand climate during the June-Dec period in 2013.

	N	Mean	SD	Min	Max
Cold stress index ($kJm^{-2}h^{-1}$)	6314	967.461	79.9	795.275	1312.99
3-day cold stress index ($kJm^{-2}h^{-1}$)	6314	968.851	62.4	807.1	1174.04

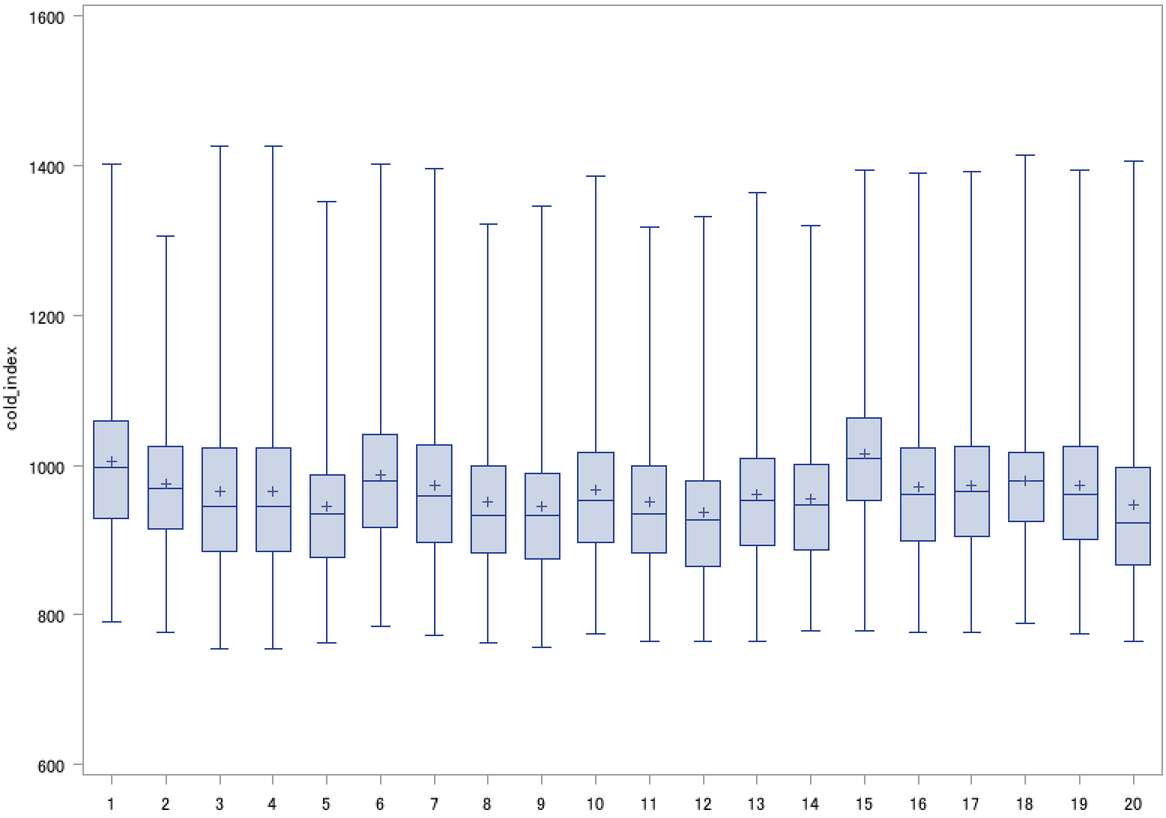


Figure 4.6: Boxplot of cold stress index for each herd for the date of first submission for the 2013 breeding season in 20 New Zealand dairy herds.

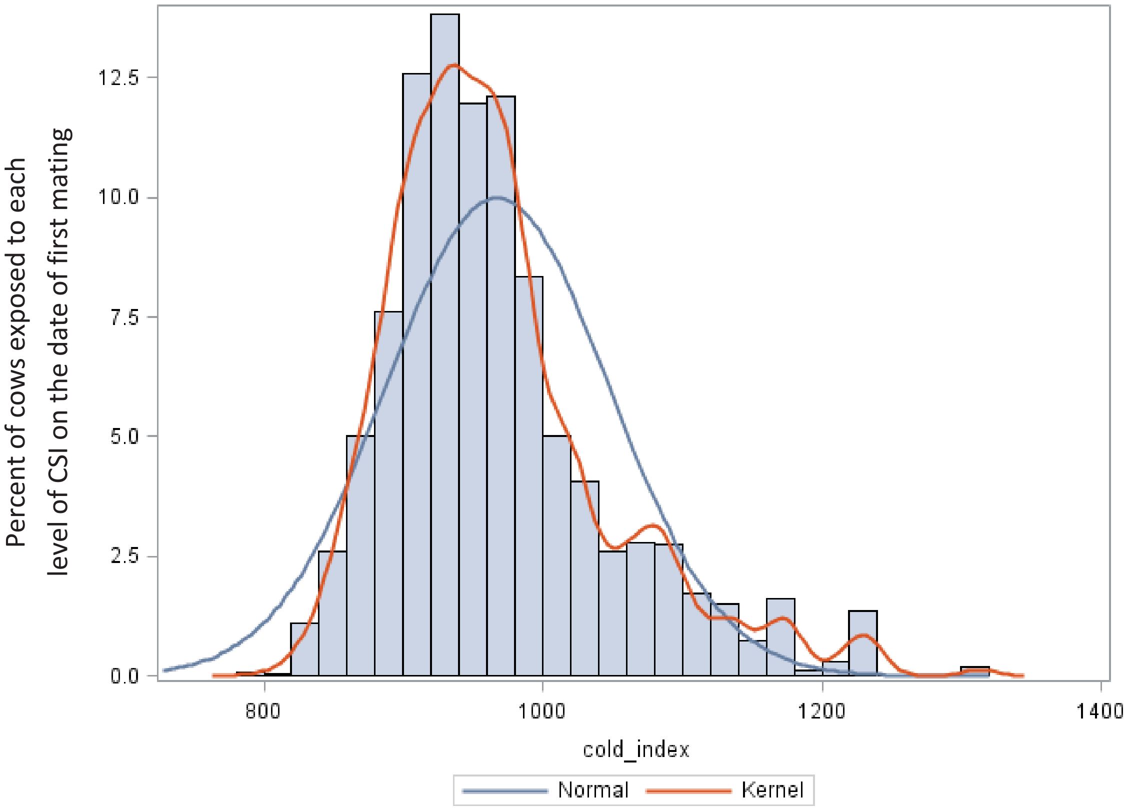


Figure 4.7: Histogram of cold stress index values for date of first submission during the 2013 breeding season in New Zealand dairy cows, showing the normal distribution and kernel density estimation.

The cold index calculates the level of stress that the cow is submitted to at any particular time. Figures 4.6 and 4.7 show the range of cold stress that the cows were exposed to on the day of first submission. The range is between 795 and 1313 $kJm^{-2}h^{-1}$, with an average of 975 $kJm^{-2}h^{-1}$, and there is a relatively normal distribution of cold stress levels. Higher values indicate a more stressful environment in relation to cold conditions (Figure 4.7).

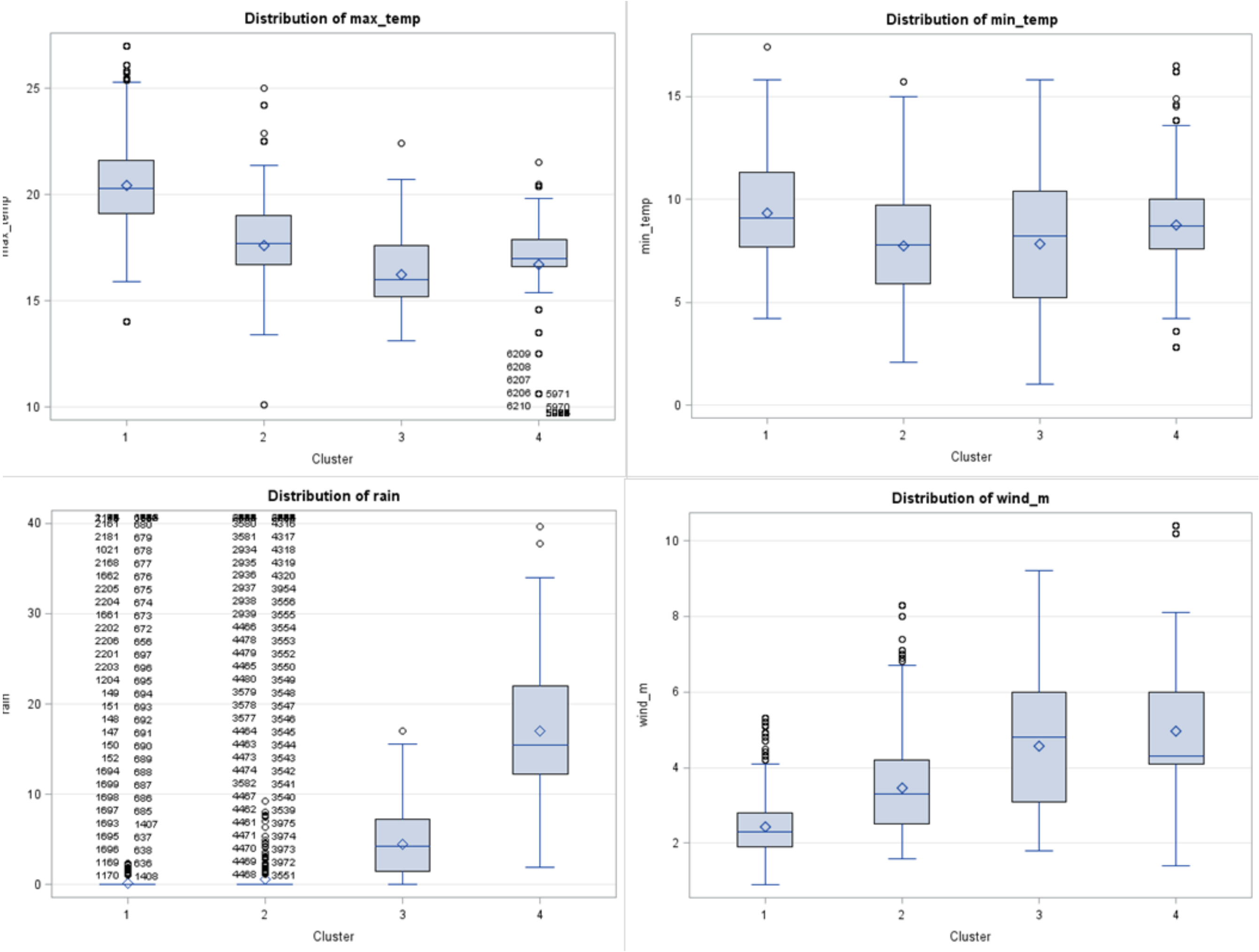


Figure 4.8: Distributions of the four key climatic traits used to calculate the cold stress index. Figures are representational of the June-December period of 2013, during which breeding takes place in New Zealand dairy herds. Top Left: maximum temperature (°C); Top Right: minimum temperature (°C); Bottom Left: rain (mm); Bottom Right: wind (ms^{-1}).

Table 4.9: Mean and standard deviation (SD) of the level of cold stress exposure for each of four clusters.

Cluster	N	Mean CSI	SD
1	2206	895.17	24.8
2	2670	963.47	20.96
3	1071	1053.52	30.68
4	367	1179.81	44.14

Figure 4.8 shows the mean and distribution of values for the key variables involved in calculating the cold stress index. The cold index has been grouped into 4 main clusters representing an increase in the level of cold stress that a cow was exposed to. Table 4.9 shows the average cold stress level in each cluster, with the trend of increasing cold stress with each additional cluster. Furthermore, there were a much larger number of cows exposed to cold stress levels representative of clusters 1 and 2 (N=2206, 2670 respectively) than the number of cows exposed to conditions representative of cluster 4 (N=367) (Table 4.10).

Both maximum and minimum temperature had little variation between each level of cold stress, with a slight downward trend for maximum temperature, whereas rain and wind showed an increase with higher cold stress levels (Figure 4.8). There was a substantial increase in the level of rainfall for cluster 4, representing 17mm of rainfall per day, in relation to the remaining 3 clusters, which all had rainfall below 5mm per day (Table 4.10).

Table 4.10: Means and standard deviations (SD) for maximum temperature, minimum temperature, rainfall and wind speed for each of the four cold index clusters. The data is representative of June-December for 2013, which is the key breeding season for New Zealand dairy herds.

Cluster	N	Max temp (°C)		Min temp (°C)		Rain (mm)		Wind (ms^{-1})	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	2206	20.42	2.31	9.34	2.25	0.08	0.33	2.43	0.83
2	2670	17.62	1.75	7.75	2.82	0.56	1.35	3.47	1.20
3	1071	16.25	1.83	7.85	3.58	4.51	3.78	4.56	1.62
4	367	16.71	2.16	8.76	3.09	17.0	8.28	4.95	1.63

As the cold stress levels increase through clusters 1-3, there is an increase in the SR21 from 60-95%, and this is then followed by a decline to 89% in the fourth cluster, which is correlated with the highest levels of cold stress in the current study (Table 4.11). Furthermore, the same trend is evident for 21d-ICR with an increase in conception rate through clusters 1-3 from 31-54% followed by a decline to 49% conception rate in cluster 4 (Table 4.11).

Table 4.11: Means and standard deviations for 21-day submission rate (SR21) and 21-day in-calf rates (21d-ICR) for each of the four cold stress index clusters, representing increasing levels of cold stress. The data is representative of June-December for 2013, which is the key breeding season for New Zealand dairy herds.

Cluster	N	SR21		21d-ICR	
		Mean	SD	Mean	SD
1	2206	0.606	0.489	0.310	0.463
2	2670	0.821	0.383	0.458	0.498
3	1071	0.954	0.209	0.547	0.498
4	367	0.896	0.305	0.498	0.501

Figure 4.9 shows the predicted milk yield for all cows on the date of first service. There is little variation between each of the four levels of cold stress, with a slight increase in milk yield of 3L for cluster 4, representing a slight increase in milk yield as the conditions become colder.

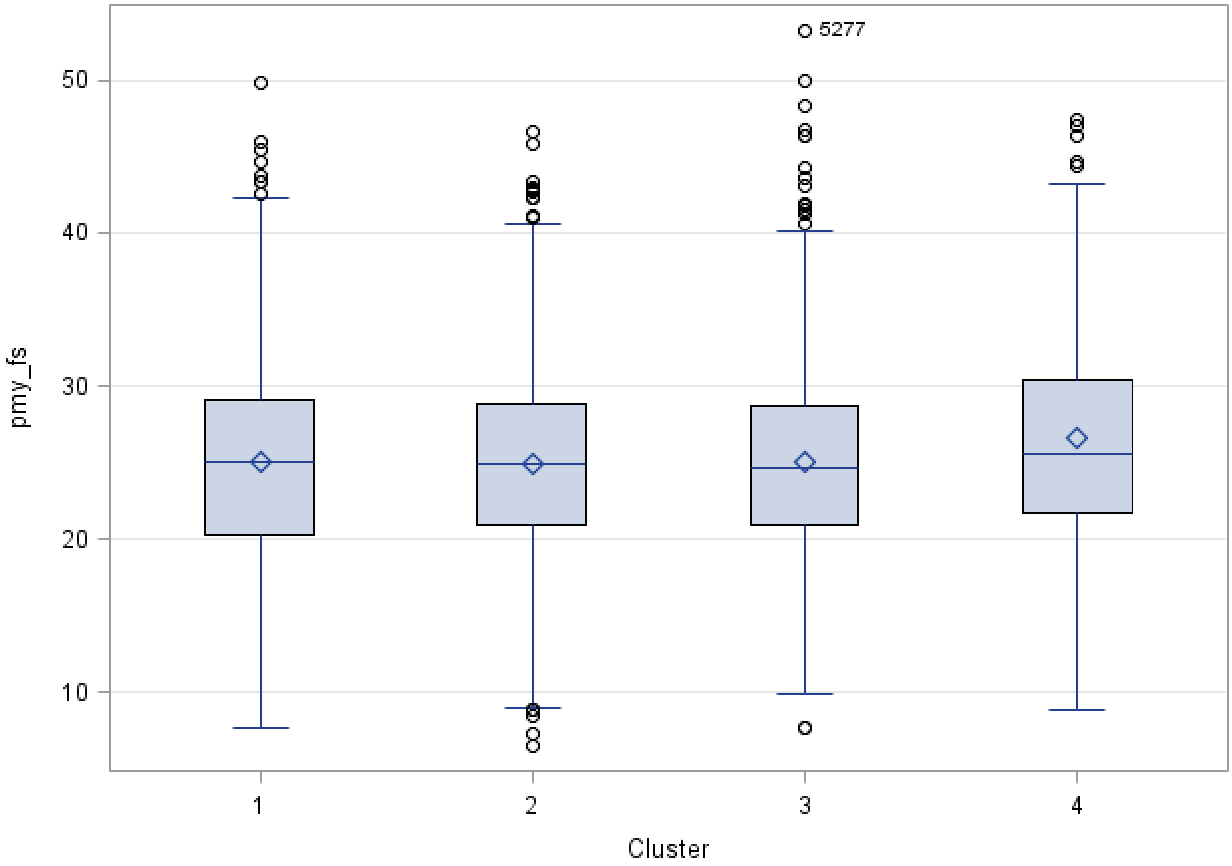


Figure 4.9: Distribution of predicted milk yield at first service (PMYFS) (L) for four different levels of cold stress that New Zealand dairy cows were exposed to on the date of first submission during the 2013 breeding season.

Chapter 5 : Discussion

The objective of this study was to find empirical evidence that supports or refutes the farmer belief that cows stop cycling during cold, wet and windy conditions. In order to do this, submission rates, conception rates (analysed as in-calf rates) and likelihood of pregnancy to first or second service were analysed against climatic variables to determine if there were any relationships that suggest this belief was valid. Results from this study show signs of a curvilinear relationship between submission and conception rates and cold stress, which is indicative of a correlation between adverse temperatures and reproductive ability in dairy cows (Bryant et al., 2007). The animals observed across the 20 herds in the present study were an accurate representation of the New Zealand dairy herd; with fertility measures at or above average for the national herd; thus, results from the present study would be representative of impacts within the national herd (DairyNZ and LIC, 2014).

5.1 Herd Statistics

The average number of days from start of breeding until first service (SBFS) was 14 days which is 3.5 days shorter than reported by Grosshans *et al.* (1997). In addition, the average number of days from start of breeding until conception (SBCO) was 21 days which is also 10 days shorter than reported by Grosshans *et al.* (1997). It is probable that the differences between results in the current study and those of Grosshans et al., (1997), are due to small increases in the fertility of dairy cows and changes in herd management over the past 15-20 years (DairyNZ, 2015). The mean 21-day submission rate was 77% and the mean 42-day submission rate was 97% which were on par with the national average of 79.9% SR21 for the same season (DairyNZ and LIC, 2014). The mean 21-day in-calf rate was 45%, similar to the 49% reported by Grosshans et al. (1997) and the mean 6-week in-calf rate was 71% which is above the national average 6-week in-calf rate of 65.6% (DairyNZ and LIC, 2014). The mean pregnancy rate to first service (PFS) of 54%, was higher than the average of 49.9% presented by Grosshans *et al.* (1997); however, the mean pregnancy to second service (PSS) of 69% was lower than the 75.9% reported by Grosshans *et al.* (1997). Again these differences in fertility traits are likely to be due to the change in genetics and management over time, with the breeding focus of the New Zealand dairy industry changing. However it is interesting to note that the pregnancy rate for the first submission has increased, but this has not resulted in an increase in overall 6-week in-calf rate, which has been a focus in the New Zealand Dairy Industry over the past 5 years (DairyNZ and LIC, 2014).

5.2 Reproductive Statistics

In New Zealand, most dairy farms operate with a single, seasonally-concentrated calving pattern (Macmillan and Moller, 1977). In a typical seasonal herd, breeding starts on a fixed date during spring and after this date, every cow which is detected in oestrus is bred (Grosshans et al., 1997). Each farm sets their own start of calving date in order to best align feed demand with feed supply, and therefore, each region differs in their relative calving and mating periods due to climatic differences throughout the country (Holmes, 2001). This creates a relatively normal distribution of mating dates in the herds analysed (Figure 4.1).

The 21-day submission rate observed in the present study is in agreement with the national dairy statistics for the 2013-14 season which indicated a 21-day submission rate of 79.9% (DairyNZ and LIC, 2014). Additionally, the average 6-week in-calf rate of 71% (Table 4.2) is in line with the top 25% of herds for the 2013-14 season, with an industry target of 78% (DairyNZ and LIC, 2014).

5.2.1 Breed, Lactation and Calving Month Differences

These results indicate that Friesian cows have a higher pregnancy rate to second service compared with crossbred cows. This result differs to previous research, which suggests that crossbred cows have increased fertility and other production traits due to heterosis, or hybrid vigour (Ferris et al., 2012; Buckley et al., 2014). These differences may have occurred for a number of reasons, including those detailed in the following discussion.

Friesian cows have a greater postpartum anoestrus interval (PPAI) than Jersey and Crossbred cows (McDougall et al., 1995). In a study by McDougall et al., (1995), there were significant interactions between stocking rate and breed for PPAI, condition score, weight and milk yield, which suggests that Friesians may be more sensitive than other breeds to changes in nutrition and the partitioning of energy which occurs at the beginning of lactation. Furthermore, research has also shown that after calving, it can take around 3 months for fertility to return to it’s optimum (Figure 5.1) (Levasseur and Thibault, 1980). Therefore, after calving, the fertility of a female continues to increase over time, with the completion of each oestrous cycle (Figure 5.1) (Levasseur and Thibault, 1980). Due to this delayed fertility, Friesian cows, with a longer PPAI may not have completed an appropriate number of oestrous cycles prior to their first service, and thus, pregnancy to first service may be relatively low, in comparison to Jersey and Crossbred cows which may have had a longer time cycling prior to the start of mating (Levasseur and Thibault, 1980). This decreased fertility at the first submission could then lead to an increased number of Friesian cows cycling and available for a second insemination, and therefore, an increased pregnancy to second service (Levasseur and Thibault, 1980).

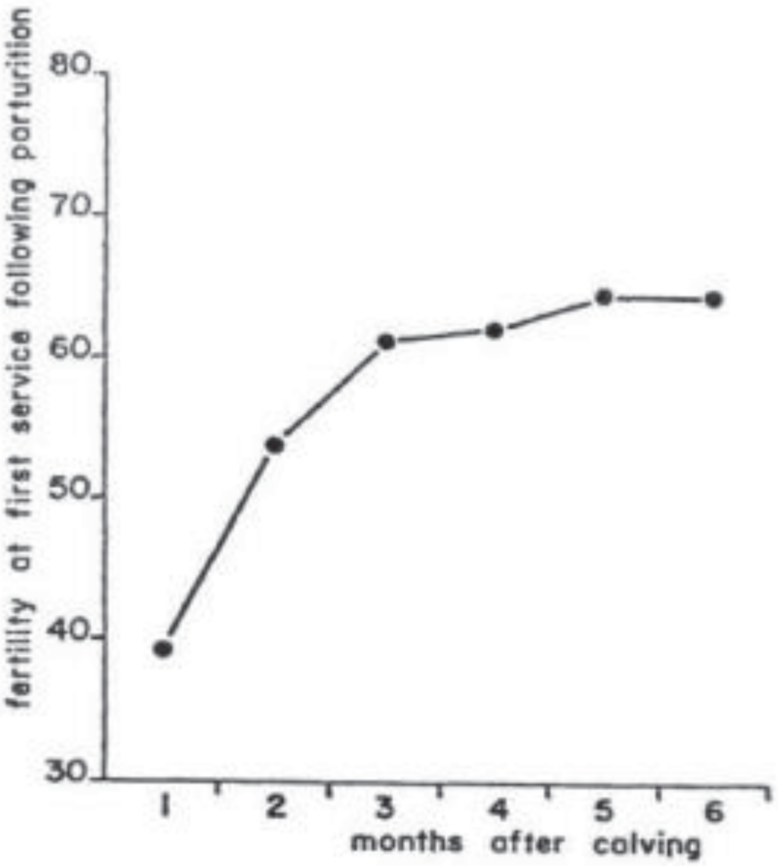


Figure 5.1: Fertility of dairy cows (number of conceptions per hundred cows mated) at first mating following calving (Levasseur and Thibault, 1980).

Younger cows (<3yo) had significantly lower submission (P=0.01) and conception rates (P<0.0004) in comparison with older cows (5yo). A study by Clarke et al., (2000) has shown that the PPAI for 2 year olds is typically much longer than mixed age cows. This same study on New Zealand dairy cows showed that 90% of 2 year olds had a PPAI of 60 days or more, whereas for mixed age cows, 50% had a PPAI less than 45 days, with 32% between 45-60 days and only 17% were longer than 60 days (Clark et al., 2000). This difference in PPAI with age may be linked with live weight and body condition of the cows at calving. All the 2 year olds from this study were recorded to be 100kg lighter than the mixed age cows. In New Zealand, heifers are still growing during their first pregnancy and lactation, and thus, some energy is still partitioned towards growth, which can hinder reproduction, and cause a longer PPAI in relation to mixed age cows who have reached their mature weight (Clark et al., 2000).

There was a difference in submission and conception rates between early- (June-July) and mid- to late-calvers (August onwards). The length of PPAI may partially cause these differences, as some of the late calvers may still be in their normal anoestrus period, recovering from calving during the beginning of the mating period (McDougall et al., 1995). However, additional research has also shown that after calving, it can take around 3 months for fertility to return to it's optimum (Figure 5.1) (Levasseur and Thibault, 1980). After calving, the fertility of a female continues to increase over time, with the completion of each oestrous cycle (Figure 5.1) (Levasseur and Thibault, 1980). Therefore, those cows that calve early have a longer period of time to complete more cycles prior to the start of mating, and hence, have higher conception rates to first service than those cows that calve later in the calving period (Levasseur and Thibault, 1980).

5.3 Meteorological Statistics

According to the National Institute of Water and Atmospheric Research (NIWA, 2014), the 2013 year was the 3rd warmest on record since the establishment of its seven station series which began in 1909. The annual climate summary for 2013 depicted an average national temperature of 13.4°C which was 0.8°C above the 1971-2000 annual average (NIWA, 2014). Figure 5.1 shows the monthly departures from the 1981-2010 averages (NIWA, 2014). In particular, July, August and November have a much higher average temperature for a large proportion of the country (NIWA, 2014).

The average maximum temperature in the current study was 17°C and average minimum temperature was 8°C during the June-December period, with low rainfall (2.9mm) and moderate wind speed (2.8m/s). These conditions correlate to an average cold stress level of 967 $kJm^{-2}h^{-1}$, which is indicative of an average daily temperature of 10°C with no wind and up to 10mm rainfall (Table 5.1). The lower critical temperature threshold for dairy cows in New Zealand for climates with strong rain and wind, suggestive of a large amount of climatic stress, was calculated to be 7.5°C, with the temperature threshold lowering with less wind and rain stimuli (Table 5.2) (Bryant et al., 2010). However, the average minimum temperature presented in this study was 8°C, which is above this threshold, and the average rainfall and wind speeds are not considered to be high (Bryant et al., 2010).

Table 5.1: Illustration of the effect of incremental changes in rain, wind and temperature for a base cold stress index (CSI) of 832 $kJm^{-2}h^{-1}$ that corresponds to a temperature of 10°C, rainfall of 0mm day^{-1} and wind speeds of $0ms^{-1}$ (Bryant et al., 2007).

Rainfall (mm day^{-1})	5	10	15	20	25	30	35
CSI ($kJm^{-2}h^{-1}$)	908	970	1021	1062	1096	1124	1147
Wind (ms^{-1})	1	2	3	4	5	6	7
CSI ($kJm^{-2}h^{-1}$)	925	1018	1111	1204	1297	1390	1483
Temperature (°C)	9	8	7	6	5	4	3
CSI ($kJm^{-2}h^{-1}$)	844	855	867	879	891	902	914
Cumulative CSI*	1016	1133	1233	1320	1397	1468	1532

*The effect on CSI of all the incremental changes in rain, wind and temperature within a column.

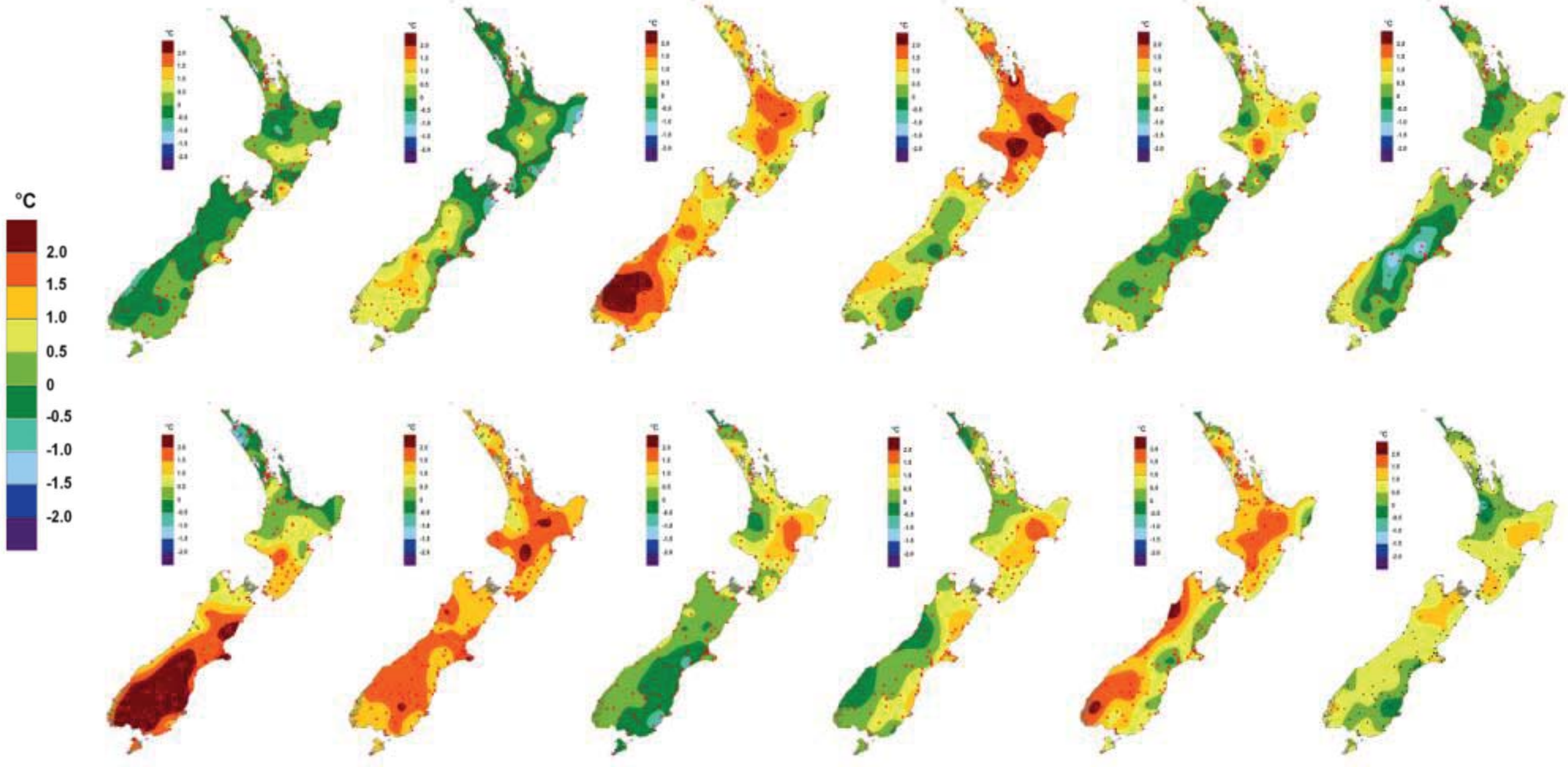


Figure 5.2: Monthly temperature departures (°C) from the 1981-2010 monthly averages. Starting in the top left with January, bottom left is July (NIWA, 2014). Temperature Scale has been enlarged on the left hand side of the Figure.

Table 5.2: Temperature thresholds for cold stress when exposed to various degrees of rain and wind for a New Zealand dairy herd fed to requirements for maintenance and pregnancy (Bryant et al., 2010).

Strong wind and rain	+7.5°C
Strong wind and no rain	+4°C
Rain and no wind	0°C
No wind or rain	-13°C

Although minimum daily temperature would be perceived as important when observing the effects of cold stress, there was no clear effect of this variable on any reproductive measures used in this study. Research has shown that average daily temperature only has a small effect on the cold stress index, with rainfall and wind speed accounting for the major changes in the level of cold stress cows are exposed to (Table 5.1) (Bryant et al., 2007). This is further supported by Figure 4.8, which suggests that rainfall and wind speed are the largest contributors to changes in the cold index for the present study. This can be seen through the larger changes in these variables when the cold index increases, in relation to the changes in minimum and maximum temperatures. Table 5.1 illustrates the effect of incremental changes in these key variables on a base cold stress index (Bryant et al., 2007). It is clear that decreasing the temperature only changes the cold index slightly, whereas small changes in the level of rainfall or wind speed have large effects (Bryant et al., 2007). Table 5.1 also shows that the maximum CSI of 1300 $kJm^{-2}h^{-1}$ from the current study, equates to approximately 20mm rainfall, $4ms^{-1}$ of wind and an average temperature of 6°C.

Maximum temperature had a negative correlation with both submission rates and in-calf rates, indicating a decrease in these parameters as the temperature increased (Table 4.3). This was further suported by the extremely low SR21 and 21d-ICR in cows in cluster 1, which was the warmest conditions that cows were exposed to (Table 4.11). It is well known that cows which are exposed to hot environmental conditions have a decrease in reproductive ability, with changes in follicular development during exposure to heat (Wolfenson et al., 1995; Chebel et al., 2007). As this was a relatively warm year, compared to the average New Zealand climate (NIWA, 2014), and due to the range of climatic regions the herds represented, there was a large variety of temperatures presented, and only a small portion of those regions exposed cows to cold stress conditions (Bryant et al., 2007). Therefore, with this decrease in submission and conception rates when the temperature increases, it is likely that there were also cows that were exposed to periods of heat stress.

5.4 Cold Stress Index

Although there has not been a large amount of research in relation to the effects of cold stress on reproductive performance of dairy cows, it has been shown that the 2 days prior to the test date had the greatest influence on milk yield in dairy cows (West et al., 2003). Furthermore, a significant association was found between the 3-day CSI and milk yield and fat:protein ratios in three different breeds of dairy cows (Bryant et al., 2007). Due to these effects, a three-day average CSI was calculated to determine the differences between three-day and single-day CSI. The three-day CSI calculated was very similar to the single-day CSI, with a slightly smaller range, as expected. However, the effects of this parameter did not differ from the single day CSI, and thereofre, only results from the single-day CSI have been analysed and discussed further.

The average daily level of cold stress that the cows in this study were exposed to was 967.5 $kJm^{-2}h^{-1}$, which is associated with conditions that lie well within a cows thermo-neutral zone, and therefore, places no climatic stress on the animals (Bryant et al., 2007). Figure 5.3 shows the effects of increased cold stress on the production of milk and milk components for three breeds of first lactation New Zealand dairy cows during the years 1990-2002 (Bryant et al., 2007). It is interesting to note that there is a curvilinear trend for three of the four parameters, which suggests that production increases in cool environments, but as it continues to cool, production is negatively affected. This may be a survival mechanism, as milk production results in a large amount of heat production, which will be beneficial in maintaining body temperature as temperatures begin to fall below the lower critical temperature. Similarly, Young (1983) showed that when temperatures fell below the lower critical temperature of the animal, metabolism increased in order to maintain body temperature through increasing the heat produced. It is likely that milk production follows a similar trend, as it is a key source of heat production for dairy cows. However, as the temperature continues to decline, the cow will reach a summit metabolism, or milk production (Figure 2.5), at which point the cow can no longer maintain body temperature, and hypothermia sets in (Young, 1983; Bryant et al., 2007). If exposed to these conditions for an extended period of time, the cow is unlikely to survive (Young, 1983). Furthermore, it has been shown that during periods of cold stress, cows tend to have lower feed intakes, and therefore, must rely on endogenous energy to maintain milk and heat production, which can result in weight loss, leaving the cow weaker (Olson and Wallander, 2002). Bryant et al. (2007), determined that milk yield started to decline at an average of 1100 and 1000 $kJm^{-2}h^{-1}$ in Friesian and Crossbred cows respectively, and that significant reduction in milk yield started to occur at 1300 $kJm^{-2}h^{-1}$. In the present study, only 25% of cows were exposed to conditions greater than 1000 $kJm^{-2}h^{-1}$ and only 1% above 1300 $kJm^{-2}h^{-1}$, which is equivalent to the 1% of days throughout the year that cows were exposed to these conditions in the study by Bryant et al. (2007).

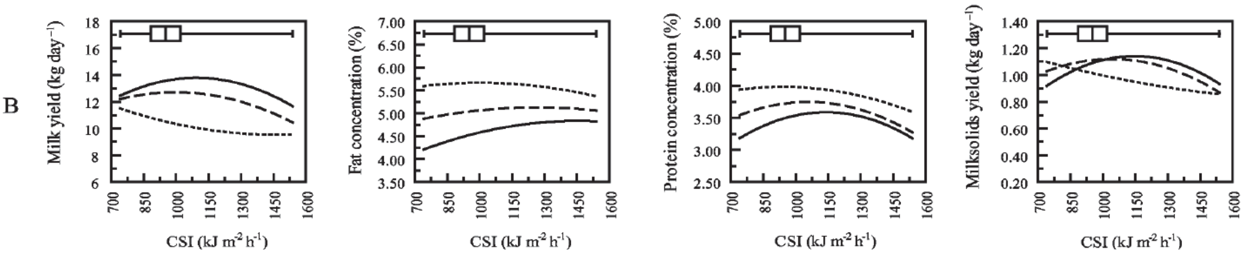


Figure 5.3: The effect of cold stress index (CSI) on yields of milk and milksolds (yields of fat plus protein), and concentrations of fat and protein in Holstein Friesian (solid line), crossbred (FxJ, dashed line) and Jersey (dotted line) cows averaged over 3-8 herd tests during their first lactation. The box and whisker plots at the top of the graphs represent the quartiles of the environmental data used in this study (Bryant et al., 2007).

The results from the current study show a small, but positive correlation for the cold stress index on all fertility parameters, including submission rates, in-calf rates and the likelihood of pregnancy to the first and second service (Table 4.4). It is probable that, as the conditions that the cows were exposed to were only in the area of 1000-1100 $kJm^{-2}h^{-1}$ or below, they are still in their increased metabolic phase, which would correlate to increased milk production and activity in an attempt to increase body temperature, and this correlates to the upward curve of the curvilinear relationship shown in Figure 5.3 (Bryant et al., 2007). Therefore, although this positive correlation is the opposite to what we expect based on farmers experiences, it may be that due to the warmer than average year, the cows were exposed to cold conditions that they were still able to function in, and further environmental stimuli such as wind and rain were required to cause a negative effect on fertility (Bryant et al., 2007).

5.4.1 Clusters

In order to further investigate the effects of cold stress, four clusters were formed on the basis of creating four distinct groups of cold stress within the data set. These four groups were then used for analyses between cows exposed to different levels of cold stress. The clusters were created in SAS (SAS, 2013), with the average level of cold stress increasing with each cluster (1-4) (Table 4.9) Figure 4.8 clearly shows the key roles of rain and wind speed on influencing the level of cold stress that the cow is exposed to, in relation to the effects of temperature. Although maximum and minimum temperature show a declining trend as the cold stress levels increase, the days are still reaching average maximum temperatures of 15-18 degrees and average minimum temperatures are within 7-10 degrees. The thermoneutral zone for dairy cows tends to be between 6 and 15°C, with variations depending on milk production, growth rates and feed intake (Nardone et al., 2006). Therefore, the average lower minimum temperature of 7°C still lies within the thermoneutral zone, and it is only on days cooler than average where cows will surpass their lower critical temperature and enter a phase of increasing production in order to maintain body temperature (Young, 1983; Bryant et al., 2007).

Furthermore, due to the seasonal systems utilised in New Zealand, the mating period often occurs in conjunction with peak lactation yields, which would lead to an increased resistance to cold stress and therefore, the lower critical temperature of the thermoneutral zone will also be lower, as the cow is producing more heat during this time. This may be associated with the increase in PMY seen in cluster 4 (Figure 4.9). This increase is consistent with the trends in Figure 5.3, with the level of cold stress (1100 $kJm^{-2}h^{-1}$) correlated to the late upward section of the curvilinear trend (Bryant et al., 2007).

Submission rates

Upon more in-depth analyses on SR21, it is evident that the positive trend between SR21 and CSI is still apparent, with an increase in SR21 from 60-95% as the cold stress index increased through clusters 1-3 (Table 4.3). However, with the more in-depth breakdown of cold stress, it is now possible to see a slight decline for the highest level of cold stress represented by cluster 4 (89%) (Table 4.11). This slight decline shows evidence that the higher levels of cold stress in this study are starting to show signs of a negative effect on the reproduction of cows. However, it is important to note that the population of cows that are exposed to these levels of cold stress is much smaller than the preceding clusters (Table 4.10), and further research and evidence would be needed to confirm this finding. However, the trend is consistent with past studies on the effects of cold stress on milk production and changes in physiology that happens when cows are exposed to stressful conditions (Webster et al., 2008). Cluster 4 has a mean CSI of 1179.81 ± 44.14 $kJm^{-2}h^{-1}$, which is consistent with the level of cold stress during which milk production began to decline in the previous study by Bryant et al. (2007). Therefore, this same trend in SR21 may be indicative of a negative relationship between cold stress and reproductive performance in dairy cows.

In-Calf Rates

The in-depth analysis of cold stress also shows the same trend for 21d-ICR. Over the first 3 clusters, in-calf rates increased from 31-54% before declining to 49% in cows exposed to the higher cold stress levels of cluster 4 (Table 4.11).

The cows in cluster 4, which are exposed to higher levels of cold stress show trends of increased milk production and decreased submission rates and in-calf rates (Table 4.11, Figure 4.9). As discussed earlier, this increase in milk production is likely to be a coping mechanism, which allows the cow to produce more heat during colder weather from the increased milk production in order to combat the unfavourable conditions. With the simultaneous decrease in submission rates and in-calf rates in these cows, it is possible that this increase in production causes a displacement of dietary energy, with more energy being directed towards milk production and maintaining body temperature, and therefore reproductive ability of the cow may be compromised, with changes in circulating hormones, particularly E2 and P4 concentrations (Broucek et al., 1991). It is suggested that during periods of cold stress, cows increase their metabolic heat production, with a higher cardiac output and redistribution of blood flow (Broucek et al., 1991). This increased blood flow would cause an increase in the catabolism of E2 and P4 in the liver, decreasing the concentration of these hormones in the blood (Broucek et al., 1991). Although a decrease in oestrogen would not stop the cow from cycling, it is the key hormone involved in the onset of behavioural oestrus and is also involved in signalling for the surge in LH which causes ovulation (Mondal et al., 2006). Therefore, if E2 levels are decreased through increased catabolism, expression of oestrus will be lower, which would lead to decreased submission rates. Anecdotal evidence indicates that during adverse weather conditions cows will orientate their bodies to stand into the wind or rain, in order to minimise the impact of the adverse climate, which has been associated with a decrease in behavioural expression of oestrus. Farmers are reliant on expression of oestrus in order to select which cows are to be submitted for artificial insemination. This theory would explain the decrease in submission rates that dairy farmers are experiencing, however, further studies would be required to confirm the theory, particularly observational studies during adverse climates, in order to confirm if this depression in oestrus expression occurs.

Additionally, a decrease in the level of P4 is associated with decreased fertility, particularly in relation to follicular development and early embryonic survival in dairy cows (Butler, 2000). Decreased concentrations of P4 can cause altered steroidogenesis in the dominant follicle, which can lead to abnormal maturation and a decline in the quality of the oocyte upon ovulation (Bilodeau-Goeseels and Kastelic, 2003). This reduced quality can cause a decrease in conception rate, or, if fertilised, may lead to abnormal growth or abortion of the embryo (Wolfenson et al., 1995). Furthermore, decreased concentrations can lead to changes to the uterine environment, making it sub-optimal for support of embryo growth, which would result in early embryonic death, and thus, loss of pregnancy (Diskin and Sreenan, 1986).

5.5 Limitations

Reproductive data was provided by CRV Ambreed, with 20 New Zealand dairy herds providing access to their herd database through the CRV Ambreed InSight Web recording application. The aim when selecting these herds was to have a representation of different climatic areas around the country, which was only partially achieved, with a good representation of North Island dairy herds, but a poor representation of South Island dairy herds (2), which are the regions that are more likely to have conditions of high cold stress.

During the mating period, farmers keep records of when each cow is inseminated. These records are then loaded onto an electronic database which also includes any other relevant information that the farmer records, including birth date, breed, calving dates, herd test results and liveweight. These records were extracted into excel files in order to create a data set including the following information for each cow: herd code, cow number, calving dates for 2013 and 2014, birth date, lactation number, breed (written as proportion of Friesian, Jersey and other), herd test results, including milk yield, fat yield, protein yield and somatic cell count, and mating submission dates.

The data being used are parameters that are regularly recorded in dairy herds. These records are being used for their simplicity and low cost, however, due to this, the data may not be as accurate as it would have been had we recorded it ourselves, or trained the farmers to ensure that all parameters were recorded for each animal. Herds were excluded if synchronisation programs were used, as this will affect the submission rates. Synchronisation programs were initially developed to improve insemination rate, largely due to the challenges faced by farmers in accurate detection of oestrus (Bissinotto et al., 2014). Synchronisation involves manipulation of hormone concentrations so that all cows are at the same stage of their oestrous cycle (Bissinotto et al., 2014). With these injections and the increased knowledge of the dairy cows oestrous cycle, farmers are able to accurately predict time of ovulation, and therefore can inseminate accordingly, a common practice known as timed insemination (Bissinotto et al., 2014). Many herds with poor submission rates utilise these synchronisation tools, as they do not have to rely on detection of oestrus for a high submission rate. However, because these programs do not require oestrus detection, they have been excluded, as climate would have no effect on the time or day of submission, and this data would skew the results.

5.6 Relevance to the industry

Results from this study suggest that there is a correlation between adverse weather conditions and reproductive ability of seasonal-calving dairy cows in New Zealand. Identifying this correlation is the first step in identifying a potential problem in the industry and therefore, allowing the industry to take further steps in order to accurately determine a solution that will help farmers minimise this issue.

Farmers currently believe that their cows stop cycling during adverse weather conditions. Previous research has shown that unless the cow is subjected to a severe case of under-nutrition or health disorder, the probability of the cow ceasing to cycle is extremely low (McNaughton et al., 2003). However, as discussed previously the alteration in circulating hormones and change in behaviour can lead to a decrease in the expression of oestrus, and also affect the follicular development of oocytes, leading to lower submission and conception rates in cows exposed to adverse conditions (Broucek et al., 1991; Wolfenson et al., 1995; Bilodeau-Goeseels and Kastelic, 2003). Therefore, there is research available that refutes the farmer belief that cows stop cycling during adverse weather conditions; however, the current study reveals that periods of severely adverse weather conditions may be linked with decreased submission rates, which would appear, to the farmers, as if the cows were ceasing cycling.

Therefore, with this information, the industry can continue to uncover information that will help farmers to minimise the effects of adverse weather conditions, through behavioural studies that will identify the extent to which oestrus behaviour is expressed, and which signs farmers are able to use as alternative identification tools during periods of inclement weather.

Chapter 6 : Conclusion

A curvilinear correlation was found between increasing cold stress levels and the submission and in-calf rates of cows in the 20 herds analysed in the current study. It was determined that when cold stress levels reached above 1000-1100 $kJm^{-2}h^{-1}$, submission and in-calf rates started to decline, with the level of decline increasing as the cold stress levels increased to 1300 $kJm^{-2}h^{-1}$, which is very similar to the thresholds that Bryant et al. (2007) discovered in relation to a decline in milk production. Results from other studies indicate that changes in hormonal profiles brought about by increased metabolic heat production and cardiac output may lead to a change in the circulating hormonal profiles which leads to a decrease in the expression of behavioural oestrus. As oestrus detection is instrumental in the reproductive performance of New Zealand dairy herds, this decrease in expression will lead to cows not being submitted when they are on heat (Broucek et al., 1991; Orihuela, 2000; Bilodeau-Goeseels and Kastelic, 2003), and can cause a longer calving spread, and a loss of income due to unexploited potential of milk and calf production (Firk et al., 2002).

Identifying the relationship between cold, wet and windy conditions, although important, is only the first step in the research process. Having identified this correlation and creating an argument that supports the reality of decreased submission rates on farm provides the platform for further research into the how and why, and most importantly, what farmers can do to minimise the effects caused during these periods of inclement weather.

The current study was limited in its evaluation of cold stress environments, as it only analysed one year, and the year studied was the 3rd warmest on record (NIWA, 2014). Although a correlation between cold stress level and submission and in-calf rates was determined, the significance of the relationship in a practical sense is low, and the threshold values remain inconclusive. Without exploring cold stress levels above 1300 $kJm^{-2}h^{-1}$, we are unable to accurately determine the full relationship and the magnitude of the effect of these environments. Furthermore, as this was purely analysis of herd data, the mechanisms behind the decrease in submission and in-calf rates were unable to be accurately identified, and are purely based on theoretical knowledge from previous studies. It would be crucial to continue research into the mechanisms that underlie this decrease in submission and in-calf rates, through collecting and profiling changes in hormones and behaviours during periods of cold exposure in relation to cows that are not placed under thermal stress, so that we may have accurate information with which to advise farmers or create tools.

With the knowledge gained from further evaluating the threshold values and mechanisms which cause a decrease in reproductive performance in New Zealand conditions, scientists will be able to create guidelines or tools for farmers that will help them to alleviate the effects of these cold stress environments. Of particular importance would be to observe cow behaviour in order to support or refute the previous findings that cows will decrease oestrus expression during exposure to cold stress. In addition, identifying the length of time that cows must be exposed to cold, wet and windy conditions before a decrease in submission or conception rates are seen, and if there are any inconsistencies during different stages of the oestrous cycle are also important.

If a decrease in oestrus expression is identified, further studies into secondary behavioural expressions of oestrus that can identify any sexual behaviours that are still expressed during these periods would provide information to farmers in order to submit cows at the correct time during adverse weather events. Furthermore, identifying whether cows utilise natural or man-made shelters during wet and windy conditions and whether utilisation of these shelters reduces the effects of cold stress on submission and conception rates may provide a simple tool for reducing the effects of cold stress when it does occur.

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