Detection of Ovulation in Dairy Cows by Twice-Daily Passive Monitoring of Reticulo-Rumen Temperature

by

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ABSTRACT

The objective of this study was to determine the ability of a passive temperature monitoring system consisting of radio frequency identification (RFID) boluses with thermistors and receiver panels to detect ovulation in high performing dairy cows. The twice-daily reticulo-rumen temperature (Trr) acquisitions of 41 early-lactation Holstein dairy cows were analyzed. The data were analyzed using two criteria: six baseline days (2d, 3d, 4d, 5d, 6d, 7d) and four temperature deviations (0.2°C, 0.3°C, 0.4°C, 0.5°C). The best criteria were chosen by selecting the baseline/deviation combination that gave the best positive predictive value (PPV). The system detected 93 true positive and 267 false positive alerts of ovulation, with a monitoring rate (MR) of 47% and a PPV of 46.2%. There were indications that the Cow Temperature Monitoring System could have a future as an ovulation detection aid, but due to the unreliability of the Wi-Fi transmission of acquisitions, more research needs to be conducted before definite conclusions can be drawn.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ACC	Accuracy
AI	Artificial insemination
BCS	Body condition score
Tb	Body (core) temperature
CL	Corpus luteum
CTMS	Cow Temperature Monitoring System
D	Day
ºC	Degrees Celsius
DEV	Deviation
DIM	Days in milk
EIA	Enzyme immunoassay
FN	False negative
FP	False positive
FPR	False positive rate
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
H	Hour
hrs	Hours
IGF-1	Insulin-like growth factor
<	Less than
L95	Lower bound of a 95% confidence interval
LCT	Lower critical temperature
LH	Luteinizing hormone
Max.	Maximum
Min.	Minimum
MR	Monitoring rate
ng/mL	Nanogram per millilitre
NEB	Negative energy balance
NPV	Negative predictive value
No.	Number
OVN	Ovulation
%	Percent
PABAK	Prevalence-Adjusted Bias-Adjusted Kappa
Prob.	Probability
P_4	Progesterone
PPV	Positive predictive value
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
RIA	Radioimmunoassay
RFID	Radio-frequency identification
ROC	Receiver operating curve
®	Registered trademark
RH	Relative humidity
SEN	Sensitivity

SPC	Specificity
Stdev	Standard deviation
TAI	Fixed Timed artificial insemination
Tb	Body Temperature
TNZ	Thermoneutral zone
TN	True negative
TP	True positive
TPR	True positive rate
Tr	Rectal temperature
Trr	Reticulo-rumen (reticular) temperature
Tv	Vaginal temperature
U95	Upper bound of a 95% confidence interval
UCT	Upper critical temperature
95%C.I.	95% confidence interval

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CHAPTER 1. Introduction

The dairy industry is one of the largest agricultural sectors in Canada. As of 2012, the industry was the fourth largest in Canada with 16 thousand dairy farms (Statistics Canada NDb). As of January 1, 2012 there were 985 thousand head of dairy cattle in Canada, not including replacement heifers, dry cows and calves. During the 2010 – 2011 calendar years, the industry generated $5.8 billion in farm receipts (Statistics Canada NDb).

The Nova Scotia dairy industry consists of 245 farms across the province with 22 thousand head of dairy cattle (Canadian Dairy Commission ND, Statistics Canada 2012). The average replacement rate in dairy herds is 45%. Although there are many different reasons why a cow may be culled, the top reason, accounting for 30.3% of culling, is reproductive failure (Statistics Canada NDa).

Infertility is a major factor limiting profitability of today's dairy farms. The three main causes for the decline in fertility are genetics, management and nutrition (Beever 2006). Failure of cows to conceive to first service increases veterinary costs and the cost of artificial insemination (AI); (Pryce et al. 2004; Gonzalez-Recio et al. 2006). The average number of lactations for Canadian Holsteins is 2.5, because cows that fail to become pregnant are culled. This short lifetime because of failure to become pregnant reduces the maximum production per cow, as well as increases the cost of replacement heifers (Beever 2006). Furthermore, infertility changes the direction of genetic selection. Too much involuntary culling limits selection for production traits, such as yield (Dechow and Goodling 2008, Fetrow et al. 2006).

A major cause of decreased fertility is the inability to detect estrus. Producers use visual behavioural monitoring of standing heat and pedometer monitoring of increased locomotory activity, but success is limited. During the 21-day (on average) estrous cycle of a dairy cow, standing heat, or the time when the cow is ready to be bred, can last, on average, around 7 hrs (Rathbone et al. 1998, Ball and Peters 2004). Detecting this period of standing heat is vital for cow fertility. While management has been made better by improving on genetics, management and nutrition, farmers have come to rely on using Ovsynch to synchronize the estrous cycle and ovulation so that insemination can be done on appointment (timed) without having to use unreliable estrus detection methods (Beever 2006, Thatcher et al. 2002). Core body temperature (Tb) monitoring has been used to detect the slight dip and then rise that occurs in cow Tb before and during estrus, respectively (Fisher et al. 2008). Various technologies, such as implantable or attached data loggers in the rectum, vagina and ear, have been used to detect these variations in temperature to help improve estrus detection (Lea et al. 2008, Bewley et al. 2008a, Redden et al. 1993). Others have shown that frequent monitoring of Tb over many consecutive days detects estrus, parturition, and illness when Tb deviates from normal over short periods of time.

A new Tb detection system consists of radio-frequency identification (RFID) transponders that contain a thermistor, and panel reader and acquisition software (DVM Systems, LLC, CO). This Cow Temperature Monitoring System (CTMS) provides a non-invasive, easy way to monitor Tb in cattle. The hypothesis is this non-invasive Tb

monitoring system could be used to determine the estrus status of the cows. This inexpensive system could remove some of the guesswork out of estrus detection, help improve herd fertility and health, reduce labour costs and provide a permanent form of identification (DVM Systems, LLC, CO).

Given the dairy industry's importance to agriculture, focus on improving dairy cow fertility is vital to improving the profitability. The objective of this research was to determine if passive monitoring of reticulo-rumen temperatures (Trr) could be used to detect ovulation in high-performing, lactating dairy cattle.

CHAPTER 2. Literature Review

2.1 The Cow Estrous Cycle

The dairy cow estrous cycle lasts an average of 20 days in heifers and 21 days in the mature cow, although the range can be anywhere from 18 to 24 days, depending on the number of follicular waves that occur during the cycle (Rathbone et al. 1998). The estrous cycle has two phases; the follicular phase, which is composed of proestrus and estrus, and the luteal phase, which is composed of metestrus and diestrus. Estrus, or Day 0, is the stage at which the cow is in "standing heat", and is ready to be bred. Estrus terminates with ovulation. During metestrus (Days 1 to 4) the vacated follicle begins to evolve into a corpus luteum (CL); (Rathbone et al. 1998). A CL is created when the granulosa and theca cells of the evacuated follicle collapse inward and blood clots in the area. The granulosa and theca cells form lutein tissue, which is responsible for the secretion of oxytocin (Sjaasted et al. 2010). In diestrus (Days 5 – 18), the mature CL synthesizes and diffuses the hormone progesterone (P_4) that prepares the uterus for pregnancy. If pregnancy does not occur, uterine $PGF_{2\alpha}$ will cause regression of the CL. The endometrium, or inner wall of the uterus, is responsible for the secretion of $PGF_{2\alpha}$ and reacts to the oxytocin secreted by the lutein tissue (Sjaastad et al. 2010). The number of oxytocin receptors increase throughout the luteal phase, which leads to an increase in $PGF_{2\alpha}$. The $PGF_{2\alpha}$ causes luteolysis, which is when the lutein tissue cells shrink and are engulfed by ovarian tissue microphages. This causes P_4 secretion to cease, and the suppression of GnRH is lifted, allowing for new follicular waves to emerge, also known as proestrus (Day 19-21); (Rathbone et al. 1998, Sjaastad et al. 2010).

The hypothalamus is the control centre for nervous and endocrine control. In relation to hormonal control, the hypothalamus responds to external and internal input, as well as on the feedback of hormones in the body, to control hormone secretion from the anterior pituitary (Sjaastad et al. 2010). Gonadotrophin releasing hormone (GnRH) from the hypothalamus causes the release of follicle stimulating hormone (FSH) and lutenizing hormone (LH) from the anterior pituitary. Follicle stimulating hormone is responsible for follicle development. A female is born with all the follicles she will ever have located on the ovaries (Sjaastad et al. 2010). The resting follicle pool, or primordial follicles, are on the ovary and are identified by having a basil lamina surrounding the oocyte. These primary follicles are sensitive to FSH and will start the follicular wave by developing into secondary follicles. Secondary follicles have developed granulosa cells and will also start forming an antrum. The follicles will develop in groups call cohorts (Sjaastad et al. 2010). The secondary follicles will be recruited and start to grow. These recruited follicles have theca cells, which secret androgens to the internal granulosa cells, which convert the androgens to estrogen. These recruited cells release low levels of estrogen and inhibin and will either go through atresia (stop developing) or will become selected follicles. Selected follicles will increase their amount of hormone production (Sjaastad et al. 2010). Estrogen and inhibin have a feedback response on the hypothalamus, which will therefore have an influence on the anterior pituitary. An increase in estrogen leads to an increase in LH and an increase in inhibin leads to a decrease in FSH. Selected follicles will also either go through atresia, or they will develop into dominant follicles (Sjaastad

et al. 2010). The dominant follicles have increased numbers of FSH receptors so they still respond to the low levels of FSH and form Graafian follicles, or the ovulatory follicle, but follicles in other stages of development will not respond to these low levels of FSH. In response to the increase in LH, fluid will build up in the maturing follicle (increasing pressure), ovary connective tissue weakens, smooth muscle contractions start in the smooth muscle and blood flow and fluid increases in surrounding tissue, which leads to the ovulation of the oocyte from the follicle (Sjaastad et al. 2010).

As mentioned above, if conception occurs, progesterone from the CL will keep further follicular waves from developing to the point where dominant follicles will occur. In the dairy cow, P_4 is best known as the ovarian hormone that maintains pregnancy. Progesterone is also responsible for increasing the growth of glands that secrete proteins to help nourish the embryo and stimulating the growth of the endometrium and stabilizing the smooth muscle to prevent early contractions (Sjaastad et al. 2010). The maintaining of pregnancy is done through the suppression of the follicular wave. Progesterone suppresses the secretion of GnRH through a negative feedback that influences the tonic and surge centres of the hypothalamus (Sjaastad et al. 2010). With GnRH suppressed, FSH is not occurring in levels that promote the development of dominant or ovulatory follicles. Production of P_4 is stimulated by LH, more specifically, the surge of LH that occurs at ovulation (Sjaastad et al. 2010). When the CL regresses, P_4 diffusion ceases, peripheral concentrations fall quickly due to the short half-life of P_4, the negative feedback on GnRH is removed and the cycle begins all over again. Therefore,

when high concentrations of P_4 occur between periods of low peripheral P_4, the cow is cycling (Fortune et al. 1991, Sjaastad et. al. 2010).

If no conception occurs, the follicular wave cycle will start anew. A wave can last four to six days and one or two waves can occur before a dominant follicle will develop, which means that there will be approximately two to three follicular waves during a single estrous cycle (Ball and Peters 2004).

2.1.1 Restoration of Estrous Cycles

A period of anestrus post-partum is important to allow the uterus to recover and support new embryo development. This process, known as uterine involution, includes tissue repair, bacterial clearance and myometrial contraction (Wathes et al. 2007). However, post-partum diseases and conditions (like NEB), peripartum disease (such as mastitis, metritis, and retained placenta), and lameness (Walsh et al. 2007) can prolong anestrus (Rhodes et al. 2003). Depending upon the severity of anestrus, there may be ovulations without expression of estrus (standing heat) that are known as "silent heats" (Rhodes et al. 2003). In very severe anestrus only small follicles are present and no ovulation occurs. This is because, when a cow is in a NEB, there is not enough energy provided to provide adequate LH production to promote follicular development (Schillo 1992, Imakawa et. al. 1986). Luteinizing hormone from the anterior pituitary is released in low amplitude high frequency pulses during estrus (Ball and Peters 2004). These low pulses will maintain until there is an increase in estrogen caused by the maturation of follicles. In response to this increase in estrogen there will be a pre-ovulatory LH surge that will

cause the ovulation of the dominant follicle (Sjaasted et. al. 2010). During a NEB, the LH pulses can be inhibited and the ovarian response can be distorted, leading to a delayed resumption of ovarian activity (Canfield and Butler 1991).

In majority of high-performing dairy cow, the anestrus period, or period from calving to first ovulation will be around 30 days (El-Din Zain et al. 1995, Opsomer et al. 1998), but can vary greatly, showing to have a range from 9 to 85 days (Savio et. al. 1990, Shrestha et al. 2004). A study by Meikle et al. (2004) reported that primiparous cows had, on average, longer anestrus periods (45 day average) compared to multiparous cows (21 day average) and that BCS had an influence on the length of the anestrus period in primiparous cows but not multiparous cows.

2.2 Factors Affecting Fertility

2.2.1 Records and the Breeding Plan

Having a good breeding plan is a vital tool of reproductive management that requires good record keeping. Traits, such as days open, days to estrus resumption, ability to conceive and time between calving and last insemination are all measures of reproductive performance. Also included are pedigree (sire and dam data), milk production records, AI and disease data. For the sire, genetic value is based on the performance (milk production, reproductive traits, conformation, etc.) of his daughters (Berglund 2008). This allows farmers to include selection for fertility in the on-farm breeding plan (Pryce et al. 2004, Pollott and Coffey 2008).

2.2.2 Management Practices

To increase the probability of cows becoming pregnant to AI, proper insemination techniques must be followed (Mee 2007) and, in addition, housing facilities must be clean with non-slip flooring to provide an environment that facilitates estrous behaviour and detection of estrus based on visual observation of standing heat (Pennington et al. 1985). Also, lighting may be controlled so that cows are exposed to a long-day photoperiod (16 to 18 hrs of light). Compared to cows exposed to a short-day photoperiod (less than 12 hrs of light), long-day light exposure can increase milk production by 2.5 kg/cow/day, which is thought to be caused by increased levels of prolactin (Dahl et al. 2000). This mimics the spring and summer months, which are ideal for breeding. With longer photoperiods, heifers will come into puberty earlier due to the increased response of LH, which allows for an earlier increase in concentrations of estrogen which remain low during pre-pubertal period (Collier et al. 2006). Cows that calve during summer months also return to estrus sooner than those that calve in the winter (Hansen et al. 1983, Hansen 1985 [as reviewed in Dahl et al. 2000]). While longer photoperiods have proven beneficial in regards to lactation and reproduction in lactating dairy cattle, there has been research that has shown that shorter photoperiods are beneficial for dry cows, allowing them to have a greater response to prolactin (the hormone responsible for lactation), and also for the immune system in calves (Collier et. al. 2006, Dahl and Petitclerc 2012). Another practice is to control the ambient temperature and humidity to avoid environmental stress (Huang et al. 2008, Nagamine and Sasaki 2008).

2.2.3 Nutrition and Negative Energy Balance (NEB)

Often, during early lactation, cows are not able to consume enough feed to meet the energy requirements needed to produce the genetic propensity for milk production, as well as maintain other body functions such as reproduction and, in the case of heifers, growth (Bradford and Allen 2008, van Knegsel 2007, Walsh et al. 2007, Reist et al. 2003). Negative energy balance (NEB) is indicated by a loss in body condition score (BCS), because the cow has mobilized body fat stores to meet the energy demand for milk production in early lactation (Leroy et al. 2008). The BCS is on a scale of 1 to 5 where 1 means that the cow is emaciated, while a score of 5 indicates that the cow is obese. An ideal BCS around the time of breeding is 3, a BCS below that indicates that the animal is without enough energy reserves to support a pregnancy (Butler 2000). After calving, there is a sudden increase in a cow's milk production, the peak of which will occur with the first 30 days post calving, which results in an increase in the energy requirements of the cows (Butler 2000). This peak in lactation not only coincides with period of NEB, but it has been shown that the timing of the cow coming out of NEB and milk production starting to decrease coincides with first ovulation (Butler 2000). Studies have shown that after calving, ensuring that the cow gets enough energy by adding fatty acid supplements and vitamins may help to decrease the severity and duration of the NEB (Pryce et al. 2004, Beever 2006, Roche 2006; Thatcher et al. 2006). Cows in NEB have abnormal endocrine function that impairs follicle and/or embryo development (Leroy et al. 2008). When cows are in a NEB, the levels of insulin in the blood lowers. Insulin is responsible for the levels of binding proteins for insulin-like growth factor (IGF-1). Insulin-like growth factor comes from the liver in response to growth hormone from the

anterior pituitary and is responsible for cell growth and development, most notably the granulosa cells of the follicle (Sjaastad et al. 2010, Royal et al. 2000). The decreased amount of binding proteins means that IGF-1 cannot bind to the follicle, thus impairing follicular cell growth (Royal et al. 2000). This impaired follicular development due to a NEB can lead to a prolonged anestrus (no follicles ovulate). Ensuring the restoration of estrous cycles is important for the development of a fertile ovulation that will result in pregnancy.

2.2.4 Ovsynch and Control of Ovulation

New developments in breeding synchronization may improve fertility by overcoming the suppression of LH and stimulating the resumption of estrous cycles (Thatcher et al. 2001). Synchronization protocols for AI at a fixed time (TAI) facilitates more rapid genetic improvement, and may also improve fertility by synchronizing ovulation so that cows can be inseminated without the need for estrus detection (Chebel et al. 2003).

One of the most frequently used commercially available synchronization protocols for dairy cattle is called Ovsynch (Thatcher et al. 2001). Ovsynch is a protocol for using GnRH (e.g. Cystorelin®, Factrel®, Fertiline® or Fertagyl®) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$); (e.g. Estrumate® or Lutalyse®) analogues so that, without estrus detection, AI can be done at the proper time relative to ovulation (Thatcher et al. 2001). The Ovsynch protocol consists of intramuscular treatments of GnRH on Day 0, $PGF_{2\alpha}$ on Day 7, GnRH on Day 9 (48h after $PGF_{2\alpha}$) and TAI on Day 10 (16 to 18 h after the second GnRH; Thatcher et al. 2001, Chebel et al. 2003, Bartolome et al. 2005). The best pregnancy rates

to the Ovsynch protocol are obtained when the first GnRH treatment causes ovulation and emergence of a new dominant follicle that ovulates to the second GnRH injection (Akoz et al. 2008, Tenhagen et al. 2004). Presynchronization strategies are sometimes used to improve the chances of pregnancy to Ovsynch TAI. For example, two doses of $PGF_{2\alpha}$ administered intramuscularly 14 days apart with the first GnRH treatment of the Ovsynch protocol administered on the 12^{th} day after the second $PGF_{2\alpha}$, has increased the chances of the cows receiving the first GnRH at the ideal times of the estrous cycle (day 5 to 12 after ovulation) to cause ovulation and synchronous emergence of a new dominant follicle (Akoz et al. 2008, Tenhagen et al. 2004). Although there have not been any studies that show that Ovsynch will improve conception rates compared to what is already achieved on farm when using visual detection, it does shorten the days from calving to A.I. and calving to conception (Akoz et al. 2008, Tenhagen et al. 2004).

2.2.5 Monitoring the Estrous Cycle of the Cow

2.2.5.1 Visual Observation and Activity Monitoring

Visual observation of standing heat was once the only method of estrus detection available to farmers and is still the preferred method in many cases. Standing heat refers to a period of time that a cow will stand to be mounted. Other visual secondary signs include licking/sniffing vulvas, chin resting (resting their chin on another animal's back), head butting, mounting and the Flehmen response (Roelofs et al. 2010, Kerbrat and Disenhaus 2004). The most commonly seen behaviours during estrus are standing heat, chin resting and sniffing/licking (Kerbrat and Disenhaus 2004, Van Vliet and Eerdenburg 1996).

In 86% of estrus events, ovulation will occur 21.5 to 33.5 hours after the onset of mounting behaviour (standing to be mounted or mounting other cows) (Roelofs et al. 2005a). One of the main downfalls of visual observation is that, to be fully effective, the time dedicated to observing cows must be consistent. While continuous, 24 hour 7 days a week observations can detect up to 73.3% of estruses (Kerbert and Disenhaus 2004); periodic observations will detect 37% of estruses (Van Vliet and Eerdenburg 1996). Continuous observation is not always practical on farm. Other factors that can influence visual observations are the number of cows in estrus at the same time and the lactation number of a cow. It has been shown that the more cows in heat together the more behavioural signs of estrus are shown. Also, primiparous cows are more active and more apt to show behavioural signs than multiparous cows (Roelofs et al. 2005a, Roelofs et al. 2005b, Van Vliet and Eerdenburg 1996).

Monitoring the activity of dairy cattle is another technique used to detect estrus. Pedometers are attached to the cow's leg (activity monitors around the neck) to detect increases in locomotory or physical activity that could be indicative of estrus. More specifically, pedometers measure the number of steps an animal takes, looking for an increase normally associated with estrus (Roelofs et al. 2005a). There is also the use of a radiotelemetry system, where pressure sensors are attached to the tail head of the animal to sense when they are mounted. Using pedometers, 75 to 80% of estrus events were able to be detected (Løvendahl and Chagunda 2010, López-Gatius et al. 2005). At-Taras and Spahr (2001) reported that the radiotelemetric monitoring of mounting behaviour had the best PPV at detecting estrus with 86.8% detection rate (meaning it was able to detect

86.8% of estruses that occurred), activity (pedometer) measurements were second with 82.6% and visual observation was significantly less efficient than both with 54.4%. One of the downfalls of this study is that there was no mention of the amount of false estrus detections that occurred with each system. Peralta et al. (2005) reported that visual observation had the highest detection rate (49.3%) compared to both a radiotelemetry detected mounting behaviour system and an activity-sensing transponder system (48.0% and 37.2%, respectively), but conception rates were higher for AI based on radiotelemetry heat-mount (24.2%) than conception rates achieved using pedometer and visual (22.2% and 18.5%, respectively) heat detection techniques. On a solitary basis, the heat-mount radiotelemetry system was the best at detecting estrus that resulted in conception after AI, but combining the visual, activity transponder and heat-mount radiotelemetry methods was actually the most beneficial since they were able to detect 80.2% of estruses when combined.

2.2.5.2 Monitoring Progesterone Concentrations

Concentrations of P_4 in plasma or serum, milk or saliva are indicative of CL function, and therefore have been used to characterize luteal (diestrus), non-luteal (estrus), or evolving (proestrus and metestrus) luteal stages of ovarian function. In plasma or serum P_4 concentrations below 1 ng/ml were indicative of no functioning CL, 1 to 4 ng/ml indicative of an evolving CL, and above 4 ng/ml indicative of a functioning mid-cycle CL (Battocchio et al. 1999). Progesterone concentrations in both blood and milk are good indicators of CL function. Compared to plasma, whole milk samples will have slightly higher and more variable concentrations of P_4. This is most likely because P_4 is a fat-

soluble hormone, but milk is easier to obtain and less intrusive than blood sampling (Colazo et al. 2008). Two ways to measure P_4 concentrations are radioimmunoassay (RIA) and enzyme immunoassay (EIA). Colazo et al. (2008) reported that when comparing RIA and two different EIAs (solid-phase EIA and direct EIA), all were able to detect P_4 concentrations in whole milk with the same precision. Radioimmunoassay is more rapid and sensitive (able to detect 0.1 ng/ml while EIA detects 0.3 ng/ml), but EIA is inexpensive and does not require the use of radioactive materials or expensive equipment like RIA. Both EIAs overestimate the P_4 concentrations in plasma and skim milk samples, direct EIA more so than solid-phase EIA.

2.2.6 Monitoring of Cow Body Temperature as an Indicator of Ovarian Function

2.2.6.1 Body (Core) Temperature (Tb) of the Dairy Cow

Several studies have demonstrated rhythms in Tb characteristic of the estrous cycle, in part due to the thermogenic effect of P_4, though the mechanisms behind this effect are not known (Kyle et al. 1998). Gil et al. (2001) reported that, in pregnant cows, Tb had increased five to twelve days after insemination. Cow Tb will be lowest just before estrus, will increase during estrus by an average of 0.5ºC, will lower again at the time of ovulation and increase again during the luteal phase (Bitman et al. 1984, Fisher et al. 2008). Kyle et al. (1998) reported that there has to be a minimum peak increase or decrease of around 0.3ºC to be an indication of estrus. This 0.3ºC deviation in Tb will occur three days before estrus. Mid-point of the cycle, another 3-day period was noted, this time with the Tb above the baseline. This coincides with what Redden et al. (1993) reported, in which estrus was indicated by an increase of vaginal temperature (Tv)

somewhere between 0.3 and 1.0ºC. They also noted the decrease in Tb from the baseline just before estrus and an increase during estrus. The maximum variation from the baseline was 0.65ºC. Rajamahendran et al. (1988) reported that, after $PGF_{2\alpha}$ injections to synchronize estrus, estrus would occur approximately 63 hours after the treatment (18 hours after a surge in LH), followed by a peak in Tv about 30 minutes later and finally ovulation, 90 hours after the injection. This study also reported that there is a direct correlation between a rise of Tv and the LH surge that occurs at ovulation and states that for Tb to be used, ambient temperature must be taken into account and that the Tb increase could be a result of the increased activity that occurs during estrus.

The average Tb of dairy cattle will be around 38.0 to 38.5ºC (Beatty et al. 2008). To maintain homeostasis of Tb, the amount of heat produced must equal the amount of heat lost from the body (Kadzere et al. 2002). This Tb homeostasis is important because the set point Tb is the optimum for metabolism (Withers 1992). Rectal temperature (Tr) above 39.4°C is considered a febrile (fever) response to infection (Benzaquen et al. 2006).

In the dairy cow, homeostasis occurs when the ambient temperature is between 5 and 25ºC. This is referred to as the thermoneutral zone (TNZ). In this zone, there is minimal physiological cost to maintaining Tb and maximum production (Kadzere et al. 2002). The TNZ ranges from a lower critical temperature (LCT) to an upper critical temperature (UCT). When the ambient temperature is below the LCT, heat production mechanisms are stimulated (e.g. shivering) to raise Tb (Kadzere et al. 2002). When the ambient

temperature is above the UCT, heat loss mechanisms are stimulated (eg. seek shade, reduce feed intake) to lower Tb (Kadzere et al. 2002). The values for these temperatures will vary, depending on species, age, diet, physiology and housing (or environment). For the dairy cow, the LCT can be anywhere from -16 to -37ºC, while the UCT is 25 to 26ºC (Kadzere et al. 2002).

Circannual (seasonal; Oster et al. 2002), circadian (24 h; Sjaastad et al. 2010), and diurnal (night and day; (Piccione et al. 2002) rhythms in Tb have been described for mammals including cattle. In the dairy cow Tb is usually highest in July and lowest in January. Daily, the temperature will be lowest at night and in the early morning and steadily increase throughout the day, reaching a peak late afternoon and then decreasing again to repeat the cycle (Bitman et al. 1984, Sjaastad et al. 2010). Lammoglia et al. (1997) reported that beef cow Tb is lowest at 0300 h, highest at 1900 h and intermediate at 1100 h. Kendall et al. (2006) supported these findings in dairy cattle, observing a rhythm in Tb, with a peak being reported in the late afternoon/late evening and the minimum being found during the early morning hours. Bitman et al. (1984) studied the entire 24-hour cycle of Tb in the dairy cow. It was reported that, over a 24 h period, a dairy cow will have a rise and fall in Tb of approximately 0.4ºC every 80 to 120 minutes.

Under moderate weather conditions and ambient temperatures, the environment does not have an effect on Tb (Verwoerd et al. 2006). Over the years, cattle have been genetically selected for increased milk production. This increase in production causes an increase in metabolic activity, more heat production and higher Tb (De Rensis and Scaramuzzi

2003). As a consequence high producing dairy cattle are more susceptible to heat stress and this can have a negative impact on dry matter intake and fertility (Collier et al. 2006, Maia et al. 2005).

2.2.6.2 Rectal Temperature (Tr) and Vaginal Temperature (Tv)

Two main sites that are usually associated with measuring the Tb of dairy cattle are the rectum and vagina (Bewley et al. 2008a, Kyle et al. 1998). However, sensors have also been attached to the inner ear to measure tympanic temperature (Lea et al. 2008). Recently, there has been more interest in using rumen temperature (Trr) as an alternative measure of Tb (Bewley et al. 2008a, Beatty et al. 2008, Small et al. 2008).

When studying the relationship between Tb and the prediction of estrus, there has been different equipment developed to help with ease of Tb measurement. Many different apparatuses developed were bulky and not practical for use due to problems with keeping the apparatus maintained and on the cow (Lea et al. 2008). The devices used to measure vaginal and ear temperatures are more exposed to the environment. This can lead to the devices being damaged, and also allows for the ambient environment to have a fast and more drastic effect on the measurements (Lea et al. 2008, Bewley et al. 2008a). There can be a lot of variation in rectal or vaginal temperature because the precision of manual measurements relies heavily on operator skills and can be very labour intensive. Also, using the thermometer requires handling the animals, which stresses the animals and can influence the measurements (Bewley et al. 2008a).

There are multiple studies that report methods for remote frequent measurement of Tb in cattle. Data loggers can be either surgically implanted or attached using a girth strap or an anal tail strap. Although relatively inexpensive, there are some problems with devices like these. For the surgically implanted loggers, there have been reactions (swelling and irritation) to the implants. The girth system needs tightening and may be expelled by the cattle (Lea et al. 2008). All of the systems are rather bulky and inconvenient, and the computer software involved can be unreliable, leading to data being lost (Aoki et al. 2005, Lefcourt and Adams 1998).

Implantable temperature sensors have also been used to monitor Tb. Fisher et al. (2008) used sensors implanted in the vagina. A bulky sensor unit needed to be attached, leading to an impractical set-up. Both of these studies used blood plasma concentrations rather than whole milk samples for P_4 analysis. Roelofs et al. (2006) noted that both milk and blood P_4 concentrations are good indicators of reproductive status.

2.2.6.3 Passive Monitoring of Reticulo-Rumen Temperature (Trr)

Newer technology for monitoring Tb in cattle uses radio-frequency identification (RFID) transponder boluses, transceiver (panel reader) and acquisition software. The bolus contains thermistors to measure the temperature in the reticulo-rumen (Trr), and a magnet separate from the electronics that can prevent hardware disease (peritoneal puncture caused by metal fines in feed). The technology is non-invasive as the transponder contains no battery, rests in the reticulum of the rumen for the life of the animal, and is activated to transmit acquisitions (Trr) when cattle pass within 1 meter of a panel reader.

The acquisitions for individual cattle are displayed on the computer screen in real-time. The RFID transponder boluses are also compatible with the mandatory Canadian Food Inspection Agency RFID ear-tag tracking system to promote food safety (Small et al. 2008). Since the boluses combine many management tools and are so easy to maintain, they are an improvement over previously used Tb monitoring techniques.

However, the temperature of the drinking water and the delay between drinking and Trr acquisitions can affect the accuracy of Trr monitoring of Tb. Bewley et al. (2008b) showed that when a cow drinks, there is a temporary but acute decrease in the Trr, with the lowest temperature occurring just after ingestion. The volume of water does not influence the change in Trr. It is the temperature of the water that will influence the degree of change in Trr. In the dairy cow, the heat produced through digestion accounts for 3 to 8% of the heat production in the rumen regardless of the amount of feed consumed (Beatty et al. 2008).

There has not been much research done with the RFID boluses because they are a fairly novel technology. Small et al. (2008) reported that the boluses were an efficient and non-invasive way to determine beef heifer Tb. Extreme variations in Tb within animals could be explained by drinking. Bewley et al. (2008a) also reported that the Trr acquired by the boluses were an effective way to monitor Tb. The RFID bolus system is a practical system available for monitoring Tb. Studies have been done to test the reliability of the transponder bolus, and the correlation between Trr and gold standard measures of Tb, Tr and Tv (Small et al. 2008, Ipema et al. 2008, Bewley et al. 2008a). No study has been

done as of yet using the RFID system to determine how well Trr monitoring can detect ovarian function in the postpartum dairy cow.

2.7 Summary and Conclusion

In summary, infertility increases the cost of primary milk production. Fertility may be improved by management strategies to prevent prolonged anestrus and failure of cows to conceive to first AI service. Frequent monitoring of Tb detects disease, estrus and calving where Tb deviates from normal for short periods of time. The new cow-temperature monitoring system offers the dairy industry continuous, tamperproof (transponder rests in the rumen protected from accidental or deliberate loss), non-invasive monitoring of Trr and protection against hardware disease throughout the animal's lifetime. The prospects for this technology to be used to improve fertility remain to be determined; the impact will depend upon how well the monitoring system detects deviations in Tb caused by production diseases, calving or estrus.

2.8 Objectives

2.8.1 Trial 1

The objective of Trial 1 was to determine if twice daily monitoring of Tv could be used to detect ovulation in high-performing, lactating dairy cattle.

2.8.2 Trial 2

The objective of Trial 2 was to determine if passive monitoring of reticulo-rumen temperatures (Trr), specifically using the CTMS, could be used to detect ovulation in high-performing, lactating dairy cattle.

2.9 Hypothesis

It is hypothesized that twice daily monitoring of Tv will be able to detect ovulation in high producing dairy cattle and that the CTMS will be able to detect ovulation through twice daily monitoring of Trr.

CHAPTER 3. Materials and Methods

3.1 Animals and Management

Cows were housed in a free stall barn (Charles Hill and Sons) located in Onslow, Nova Scotia, Canada. Cows were fed a total mixed ration of 50% corn and 50% grass and free choice water was available from two water troughs located at either end of the barn. Cows were exposed to approximately 18 hrs of light a day. Morning milking occurred, on average, at 04:07h (± 0.01) and afternoon milking, on average, at 15:17h (± 0.01). Cow's ages ranged from two years to seven years of age. All procedures were reviewed and approved by the Nova Scotia Agricultural College Animal Care and Use Committee as being in accordance with the Canadian Council on Animal Care guidelines (Canadian Council of Animal Care 2009).

3.2 Trial 1 – Preliminary Trial

3.2.1 Animals and Management

Between March 25, 2009 and May 11, 2009, 19 Holstein cows from a herd of 150 were used for this trial. Sixteen of the cows were primiparous and three were multiparous (see Appendix A for trial cow information).

Farm staff performed visual observations for estrus at least twice a day, with more occurring as opportunity presented itself. Visual observations were considered positive for estrus if there was a subsequent AI that resulted in pregnancy. Visual observation was

combined with activity monitors attached to neck collars (BouMatic®) on the cows. Visual and activity data were used in combination to identify estruses.

3.2.2 Temperature Data and Milk Sample Retrieval

Vaginal temperatures (Tv) of the cows were measured with a standard thermometer (Kaz Inc., Model 4965CF) inserted into the vagina twice daily, taken as they left the milking parlour after the morning milking starting on March 25, 2009. As cows left the milking parlour, they were identified by numbered neck collars and guided into nearby stalls so that Tv could be taken. This was done so that Tv was obtained at approximately the same time every day. Ambient temperature data was collected from Environment Canada (Debert, NS weather station).

Milk samples were taken every other day starting on April 3, 2009 until the end of the sampling period. The end of the period was May 11, 2009 unless the cow was culled before that time. Each milk sample was taken after an experienced worker sanitized (pre dipped with iodine) and stripped the teats (hand milked a couple of times to remove dirt from teat canal and stimulate milk let down) and before the milking machine was attached. Plastic, 2 oz flip-top vials (Capitol Vial Inc., Cat. No. 02CL) were used. Each vial contained a bronopol preservative tablet. Samples were stored at -20°C, later thawed in a water bath set to 55°C and divided into three separate aliquots in 8 mL disposable, round bottom, plastic culture tubes (Fisher Scientific, Cat. No. 211-0071). The aliquots were then stored at -20°C.

3.3 Trial 2

3.3.1 Animals and Management

Between October 19, 2009 and January 30, 2010, 41 Holsteins from the same herd as Experiment 1 were used. Eighteen of the cows were primiparous and 23 were multiparous (see Appendix B for trial cow information). The cows were managed under the same system as mentioned in Experiment 1.

3.3.2 Bolus Administration and Cow Temperature Monitoring System Set-Up

Radio-frequency identification (RFID) transponder boluses (Figure 1) were administered to 32 Holstein cows on October 19, 2009 and 9 Holstein cows on November 22, 2009. Cows were selected based on calving date, so that as many complete estrous cycles as possible could be monitored. An experienced handler administered boluses per os.

The CTMS was set up at the end of each milking parlour exit ally (Figure 2). The drive panel, which is responsible for activating the bolus, was mounted on the right side of the cows as they left the ally and the receiver panel, which receives the data from the bolus, was mounted on the left. Both panels were 42 cm from the ground (measured from the bottom of the panel) and 42 cm from the end of the ally (from side closest to ally end). The panels were mounted on already existing metal stall walls. The panels were wired to a control box located above the panels, which powered the panels and sent data information from the panels to the computer by Ethernet connection. The control box

was connected to a router, which sent the data to a personal computer located in the barn office (Figure 3). The data was automatically uploaded to the panel software (DVM Systems TempTrack®, version 1.2) on the computer.

3.3.3 Temperature Data and Milk Sample Retrieval

Temperature data was downloaded four to five times weekly from the computer. The CTMS was checked for functionality at each download time. Ambient temperature data was collected from Environment Canada (Debert, NS weather station).

Milk sampling occurred twice weekly (every Tuesday and Friday) during the sampling period per recommendation of Kyle et al. (1998). Each milk sample was taken after an experienced worker sanitized (pre dipped with iodine) and stripped the teats and before the milking machine was attached. Plastic, 2 oz flip-top vials (Capitol Vial Inc., Cat. No. 02CL) were filled to the 2 oz fill line already on the vials. Each vial contained a bronopol preservative tablet. Milk samples were separated into three separate aliquots occurred directly after sampling. Samples were stored at -20ºC until analysis.

3.4 Progesterone Analysis

Radioimmunoassay of skim milk progesterone concentrations was used as the gold standard for identifying ovulation. The milk samples were analyzed in duplicate at the University of Saskatoon (Endocrine Lab, WCVM Building) using a solid phase RIA (Siemens Coat-A-Count, TKPGX). The assay sensitivity (lowest amount of P_4 able to be detected) was 0.02 ng/mL. Standards ranged from 0 ng/mL to 40 ng/mL. For trial one,

the intra-assay variation was 9.8% and the inter-assay variation was 15.3%. For trial 2, the intra-assay variation was 10.3% and the inter-assay variation was 15.1%.

3.5. Data Analysis

3.5.1. Trial 1

Data from cows that were confirmed pregnant (by veterinary palpation) were removed after the confirmed pregnant date, as well as any abnormal data attributed to causes such as illness. Average Tv for morning and afternoon were calculated. Baselines (2 – 7 days) and deviations (0.3 to 0.5 °C) from baseline were also calculated. For example, if looking at a two-day baseline and a 0.3°C deviation, the present temperature reading is compared to the average of the previous two days and if an increase in temperature of at least 0.3°C is seen it is considered an indication of ovulation. Each baseline day and deviation combination had the ovulation events classified as true positive (TP), false positive (FP), true negative (TN), and false negative (FN) in relation to the detection of ovulation for each seven-day observation period for each cow. If P_4 determined no ovulation it was classified as a negative and if Tv indicated to increase in temperature (or no event) then that seven-day period of Tv measurement was classified as a TN. This allowed for the following calculations:

True Positive Rate (TPR (Sensitivity)): $\frac{TP}{TP + FN}$

Specificity: $\frac{TN}{FP + TN}$

False Positive Rate (FPR): 1 – Specificity

$$\text{Accuracy: } \frac{TP + TN}{TP + TN + FP + FN}$$

$$\text{NPV: } \frac{TN}{TN + FN}$$

For each of the combinations of criteria, the TP, FP, TN, FN from each cow were added (for example, the number of TP, FP, TN, FN for 2d, 0.2°C deviation from all cows), which gave 24 events. The TPR and FPR for each combination of criteria were then graphed against each other to give the receiver operating curve (ROC), which allowed selection of the criteria that gave the best TPR while still maintaining a low FPR. The McNemar's test was done to check for bias and when bias was found it was taken into account using the Prevalence-Adjusted-Bias-Adjusted-Kappa (PABAK) test for agreement (Table 1).

3.5.2. Trial 2

For each cow, the temperature and P_4 data were graphed to identify periods where temperature data was retrieved for an entire estrous cycle and overlapped with P_4 data so that analysis for ovulation detection could occur. Animal dropouts (non-functioning boluses, no return to estrus) and individual dropouts (temperatures below 37.5ºC, attributed to drinking) were removed from the analysis, as well as any abnormal data attributed to causes such illness.

From the data acquired from the CTMS software, Trr averages for both morning and afternoon on each day of the trial were calculated. Calculations were then done to

establish baseline temperatures and deviations from these baselines. Baselines were done on a two-day, three-day, four-day and five-day basis. For example, for a three-day baseline temperature, the average of the temperatures of the three days before the Trr temperature point was calculated. The current Trr average temperature was then subtracted from the baseline temperature to give a current Trr deviation from the baseline.

Deviations of 0.2, 0.3, 0.4, and 0.5 were used for this study. Based on the P_4 profile (as an indicator of stage of cycle) deviations were either considered TP (a correct indication of ovulation) or a FP (an incorrect indication of ovulation). The sum of the TP and FP for each baseline and deviation combination was calculated for each cow, and then an overall sum of all the cows was done. This allowed for a positive predictive value (PPV) and negative predictive value (NPV) to be calculated using the following calculation:

$$\text{PPV: } \frac{TP}{TP + FP}$$

Monitoring rate (MR) was also calculated by dividing the obtained number of useful data acquisitions to the number of potential data acquisitions.

Table 1: Kappa agreement scale used for the comparison of vaginal and progesterone detection of ovulation methods in dairy cattle (adapted from Viera and Garrett 2005).

Agreement	Kappa
Poor	<=0
Slight	0.01 to 0.20
Fair	0.21 to 0.40
Moderate	0.41 to 0.60
Substantial	0.61 to 0.80
Excellent	0.81 to 1.00

Figure 1: Radio-frequency identification (RFID) transponder bolus containing a thermistor used to monitor reticulo-rumen temperature as part of the Cow Temperature Monitoring System.

Figure 2: Drive panel (right) and receiver panel (left) for the Cow Temperature Monitoring System set up at the exit ally of the milking parlour at Charles Hill and Sons Farm.

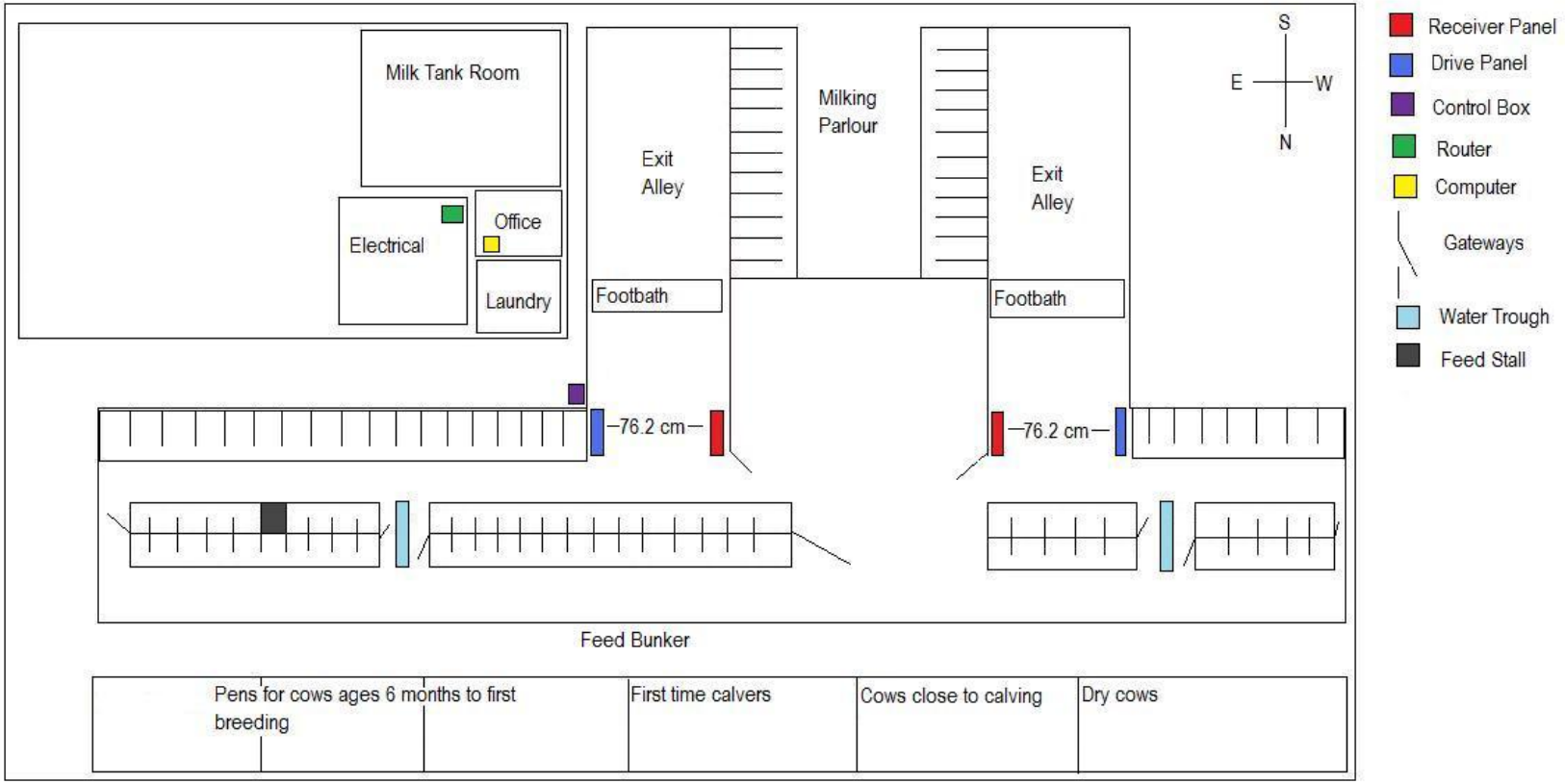


Figure 3: Aerial diagram of Charles Hill and Sons freestall dairy barn and 24 cow side-by-side milking parlour showing the set up of the Cow Temperature Monitoring System.

CHAPTER 4. Results

4.1. Trial 1

Ambient temperature during the trial had a maximum of 23.6ºC and a minimum of –8.3ºC, with a mean temperature of 7.1ºC. There was one possible day where heat stress could have occurred, if the relative humidity (RH) was above 75% (West, 2009), though homeostasis of Tb is usually maintained up to 25ºC (Kadzere et al., 2002). Due to the cooling measures taken in the barn (fans, temperature controlled blinds), it is not likely that RH was that high, and therefore the chance of heat stress was minimal.

Of the 19 cows used in trial 1, 23 estruses were detected from 16 of the cows. These were confirmed to be true estruses based off of progesterone concentrations. Of the three cows that showed no indication of estrus, two were pregnant and one was considered to be acyclic. Examples of the data retrieved for Trial 1 can be seen in Figure 4.

The ROC curve (Figure 5) identified the best criteria as the 4d baseline and a 0.3ºC deviation, with a TPR (sensitivity) of 82% and a FPR of 20% (Table 2). When bias was found using McNemar's it was corrected using a PABAK and a value of 0.61 was obtained, showing a substantial agreement between Tv and P_4 (Table 3). For P_4 and visual detection, the PABAK produced a result of 0.33, showing fair agreement between the two (Table 3).

When Tv monitoring of ovulation is compared to the visual observation and activity combination used on-farm to detect estrus, visual observation had a much better FPR

(4%) compared to twice daily Tv acquisitions (20%) (Table 2). For TPR, twice-daily Tv had a rate of 82%, which was an improvement over the 74% TPR visual observation obtained but not as good as the 100% TPR P_4 measurement obtained ($P < 0.05$). The PPV for Tv was 50%, indicating a 50% chance that the TP detected were actually true detections of ovulation, which is not as good as visual and P_4, which had PPVs of 85% and 100% respectively. The NPV for Tv monitoring was found to be 95%, which means that it can be 95% sure that identifications of no ovulation are true. The accuracy and specificity for Tv monitoring were 81% and 80% respectively (Table 2).

4.2. Trial 2

Ambient temperature during the trial had a maximum of 17.7ºC and a minimum of –19.5ºC, with a mean temperature of 7.1ºC. There were no days that indicated possible heat stress.

Due to malfunctions with the CTMS there was loss of data during the trial due to the Trr readings from the boluses not being sent to the computer. The loss of data was attributed to the panels being first set up with a wireless connection from the router to the computer that collected the data. To remedy the unreliability of the Wi-Fi connection, the router and computer were hardwired. This improved the data collection, although there were still periods of lost data and seven boluses never regained functionality.

Of the 41 cows, 12 were confirmed pregnant by veterinary palpation before the CTMS started functioning and seven had non-functioning boluses, rendering the data from 19 of

the cows unusable. Seven of the cows were confirmed pregnant at some point during the trial and had partial data and 15 cows had full Trr data, leaving 22 cows with the possibility of ovulations with both Trr and P_4 data.

The average Trr of the rumen was found to be 37.9ºC (Stdev 0.47) (Table 5). The average possible number of Trr readings was 208 and the average actual number of readings was 104. The average monitoring rate (MR) for these was 47%. The average expected count for P_4 was 17 while the average actual count was 16, giving an MR of 96% (Table 4).

Reticulo-rumen temperature and P_4 data were graphed to compare trends and identify periods of possible detection by the CTMS (Figure 6, Figure 7 and Figure 8). After graphing, it was found that there were 14 P_4 confirmed ovulations from 12 cows that coincided with Trr data that covered a seven-day period with temperatures above the cut off of 37.5°C. Cows were sampled for 104 days and since the average estrous cycle lasts approximately 21 days, there was a possibility to detect approximately 4 ovulations per cow. For all 41 cows, there was a possible 164 ovulations to detect. For the 22 cows with data, that would leave 88 ovulations for possible detection. Of those 88 ovulations, 14 of them coincided with Trr data (and occurred among 12 of the cows).

The combination of criteria that had the best PPV was 5d and a 0.3ºC deviation, which had a PPV of 46.2%. This means there is a 46.2% chance that the TP detected were actually true detections of ovulation (Table 5).

It should be noted that, for all baselines, deviations of 0.2ºC and 0.3ºC had higher FP rates then the 0.4ºC and 0.5ºC deviations (Table 6). This was due to the fact that there was more data available for the shorter baseline days than the longer ones, giving more opportunity for more FPs to be detected.

Table 2: Statistics for selection of the best criteria for detecting ovulation using twice daily monitoring of vaginal temperature in dairy cattle

Baseline (d)	Dev (°C)[a]	TP[b] Correct OVN[c]	FP[d] Incorrect OVN	TN[e] Correct No OVN	FN[f] Incorrect No OVN	Total Obs.	TPR[g]	FPR[h]	SPC[i]	ACC[j]	PPV[k]	NPV[l]
3	0.3	17	25	67	5	114	0.77	0.27	0.73	0.74	0.40	0.93
4	0.3	18	18	74	4	114	0.82	0.20	0.80	0.81	0.50	0.95
5	0.3	16	14	80	4	114	0.80	0.15	0.85	0.84	0.53	0.95
3	0.4	12	13	81	8	114	0.60	0.14	0.86	0.82	0.48	0.91
4	0.4	11	12	82	9	114	0.55	0.13	0.87	0.82	0.48	0.90
5	0.4	11	13	82	8	114	0.58	0.14	0.86	0.82	0.46	0.91
3	0.5	8	8	86	12	114	0.40	0.09	0.91	0.82	0.50	0.88
4	0.5	7	8	86	13	114	0.35	0.09	0.91	0.82	0.47	0.87
5	0.5	8	7	87	12	114	0.40	0.07	0.93	0.83	0.53	0.88
Visual Observation		17	3	80	6	100	0.74	0.04	0.96	0.97	0.85	0.93
Progesterone Concentration		23	0	5	0	28	1.00	0.00	1.00	1.00	1.00	1.00

[a] DEV – Deviation from baseline temperature
[b] TP – True positive (true indication of ovulation)
[c] OVN – Ovulation
[d] FP – False positive (False indication of ovulation)
[e] TN – True negative (True indication of no ovulation)
[f] FN – False negative (False indication of no ovulation)
[g] TPR – True positive rate, ability to identify positive results. Also called sensitivity (TP/(TP+FN))
[h] FPR – False positive rate, proportion of ovulations detected when not present (1 – specificity)
[i] SPC – Specificity, ability to identify negative results (TN/(FP+TN))
[j] ACC – Accuracy ((TP+TN)/(TP+TN+FP+FN))
[k] PPV – Positive predictive value, proportion of positive test results that are true (TP/(TP+FP))
[l] NPV – Negative predictive value, proportion of negative test results that are true(TN/(TN+FN))

Table 3: McNemar's, Kappa and Prevalence-Adjusted-Bias-Adjusted-Kappa (PABAK) for the agreement between vaginal temperature monitoring and methods to detect ovulation in dairy cattle.

	McNemar's	Kappa	PABAK	Probability
Progesterone	0.003	0.50	0.61	<0.001
Visual	0.107	0.11	0.33	<0.001

Table 4: Simple statistics for reticular-rumen temperature and progesterone monitoring of ovulation in dairy cattle.

	Expected[b]	Observed[c]	MR[d]	Mean	Minimum	Maximum	Stdev
Trr[a]	208	104	47%	37.91	36.51	40.05	0.47
P4	17	16	96%	0.89	0.02	2.66	

[a] Reticulo-rumen temperature
[b] Expected number of acquisitions
[c] Number of actual acquisitions obtained
[d] Monitoring rate ((No./Expected)*100)

Table 5: Sensitivity and positive predictive value for all baseline and deviation combinations of criteria for detecting ovulation by the Cow Temperature Monitoring System in dairy cattle.

Baseline (d)	Deviation (°C)	TP	FP	PPV
2	0.2	10	30	25.0
	0.3	9	20	31.0
	0.4	5	10	33.3
	0.5	1	14	6.7
3	0.2	11	26	29.7
	0.3	5	32	13.5
	0.4	2	9	18.2
	0.5	3	10	23.1
4	0.2	7	25	21.9
	0.3	10	21	32.3
	0.4	3	11	21.4
	0.5	2	8	20.0
5	0.2	5	24	17.2
	0.3	12	14	46.2
	0.4	4	10	28.6
	0.5	3	12	20.0

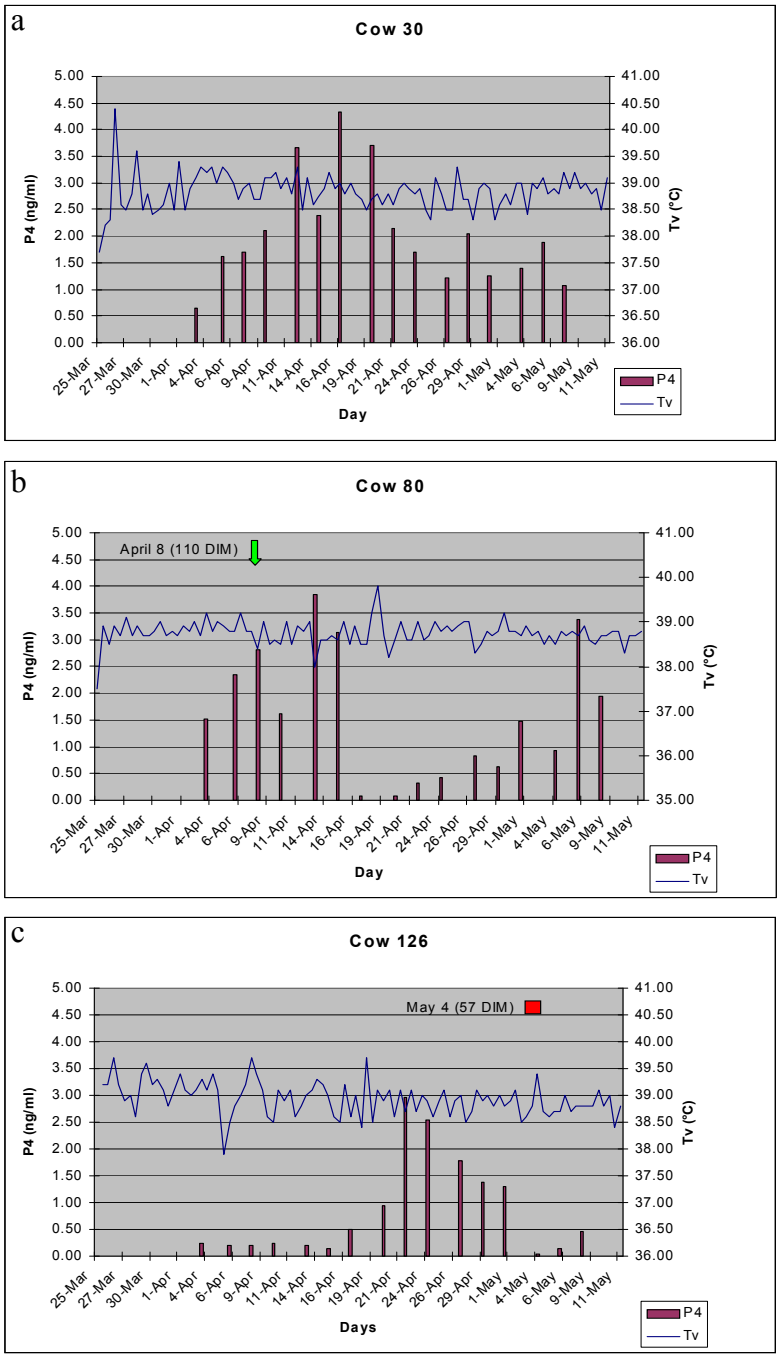


Figure 4: Representative data for individual cows in Trial 1 showing vaginal temperature (Tv) and progesterone (P4) concentrations for cows that were a) pregnant b) confirmed pregnant to artificial insemination (AI) (green arrow represents AI date with conception) and c) subjected to AI but not pregnant (red box represents AI date with no conception).

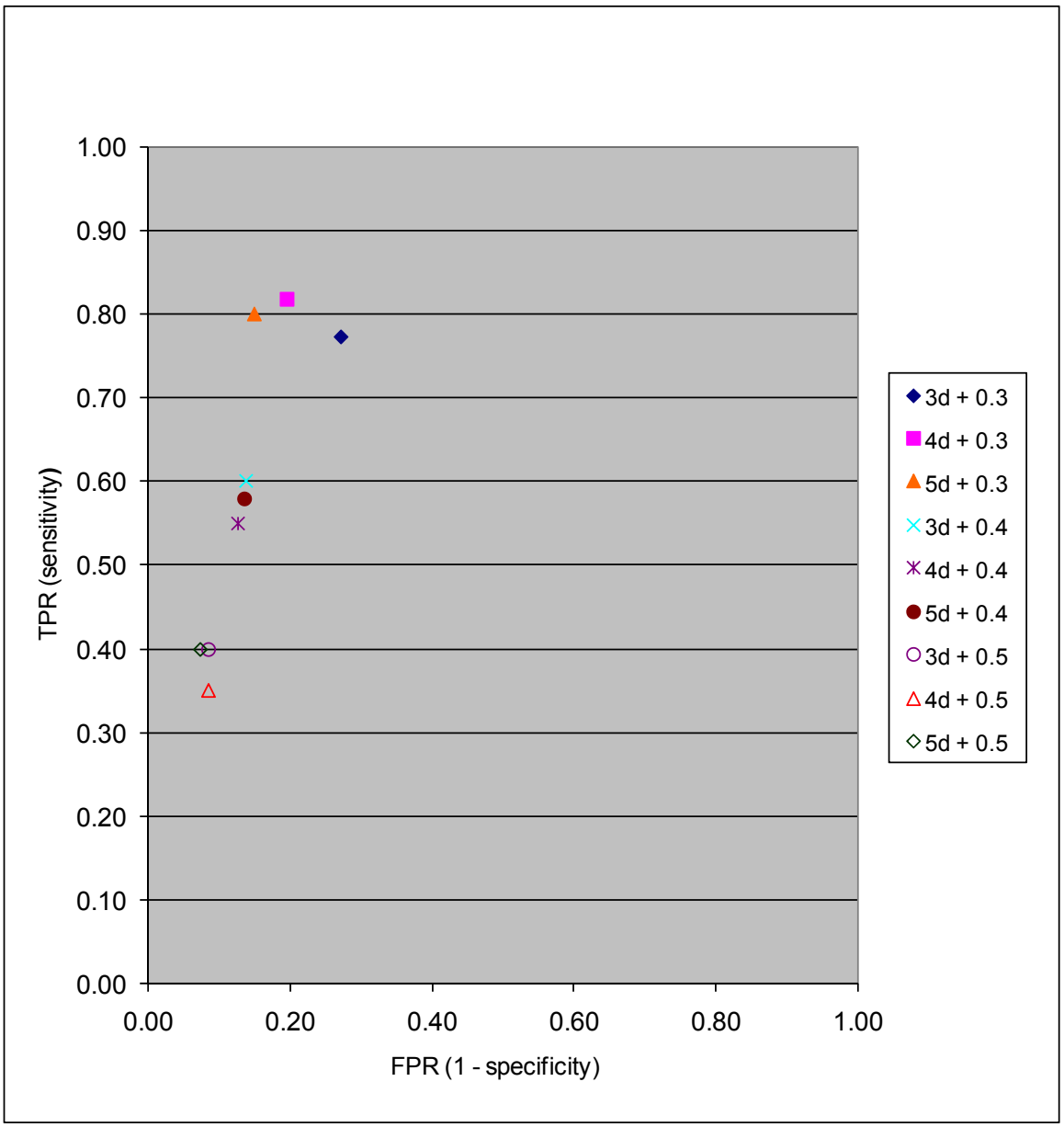
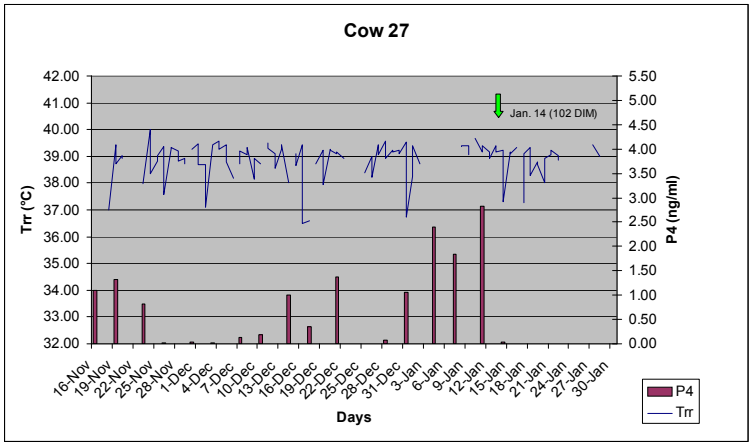
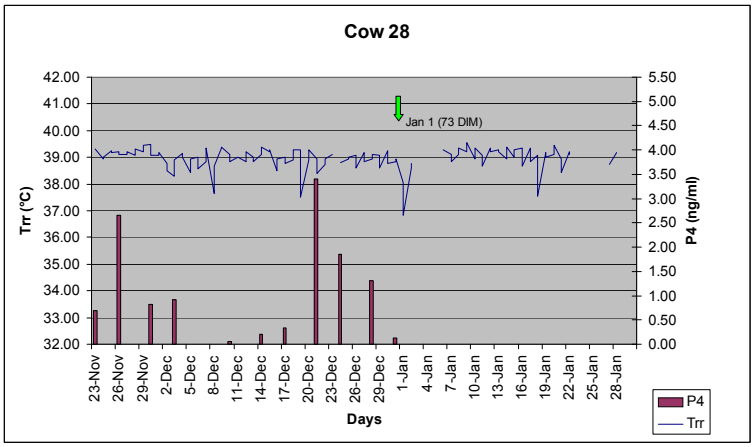


Figure 5: Receiver Operating Curve for the combination of criteria for Tv detection of ovulation in Trial 1.





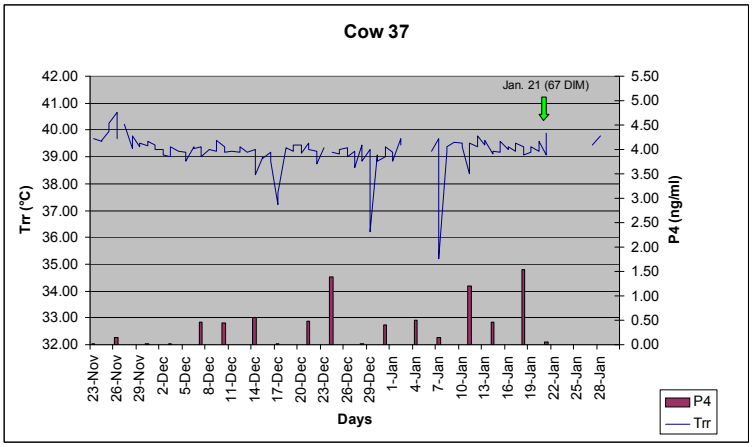


Figure 6. Representative data for individual cows in Trial 2 showing reticulo-rumen temperature (Trr) and milk progesterone concentration (P4) for cows that were confirmed pregnant to artificial insemination (AI). Green arrows represent AI dates with confirmed conception.

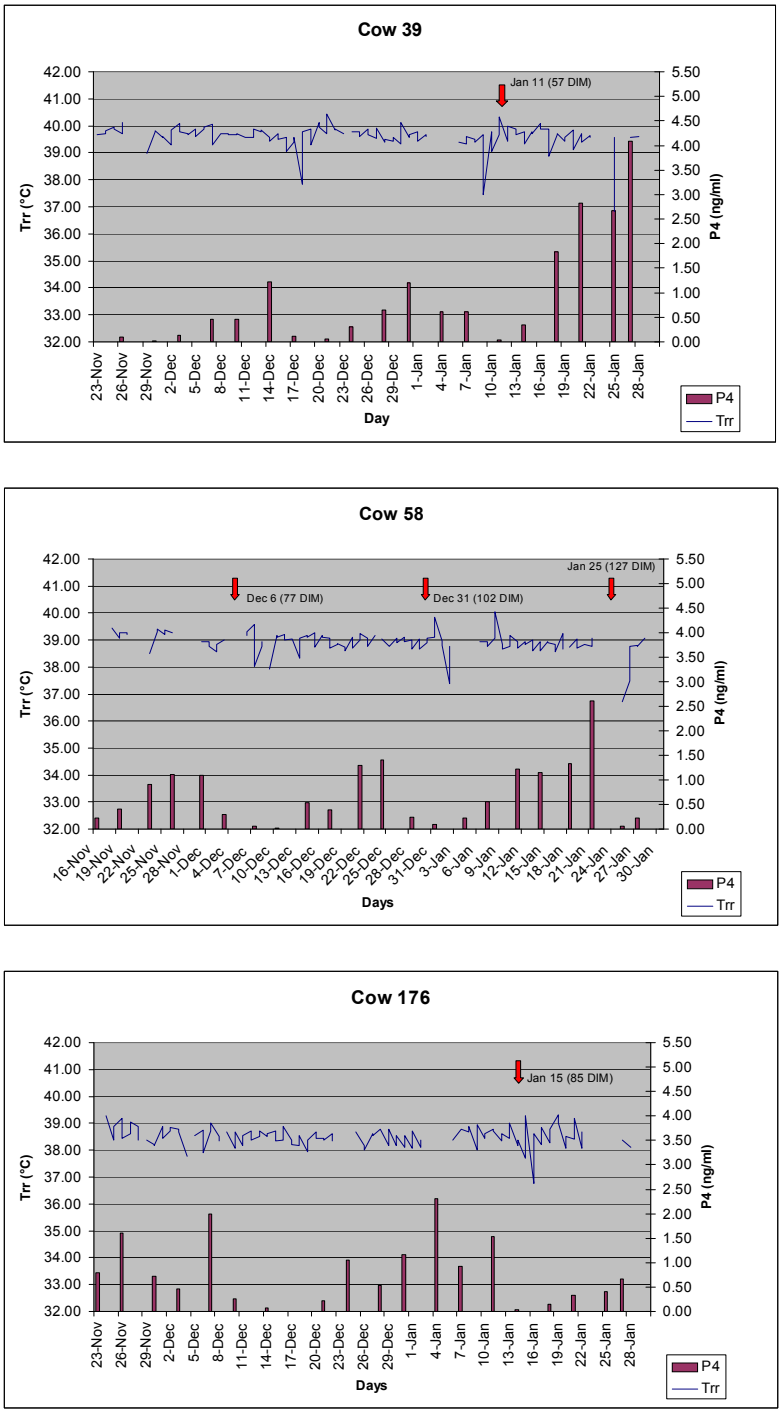


Figure 7. Representative data for individual cows in Trial 2 showing Trr and P4 for cows that were not confirmed pregnant due to AI. Red arrows represent AI dates without confirmed conception.

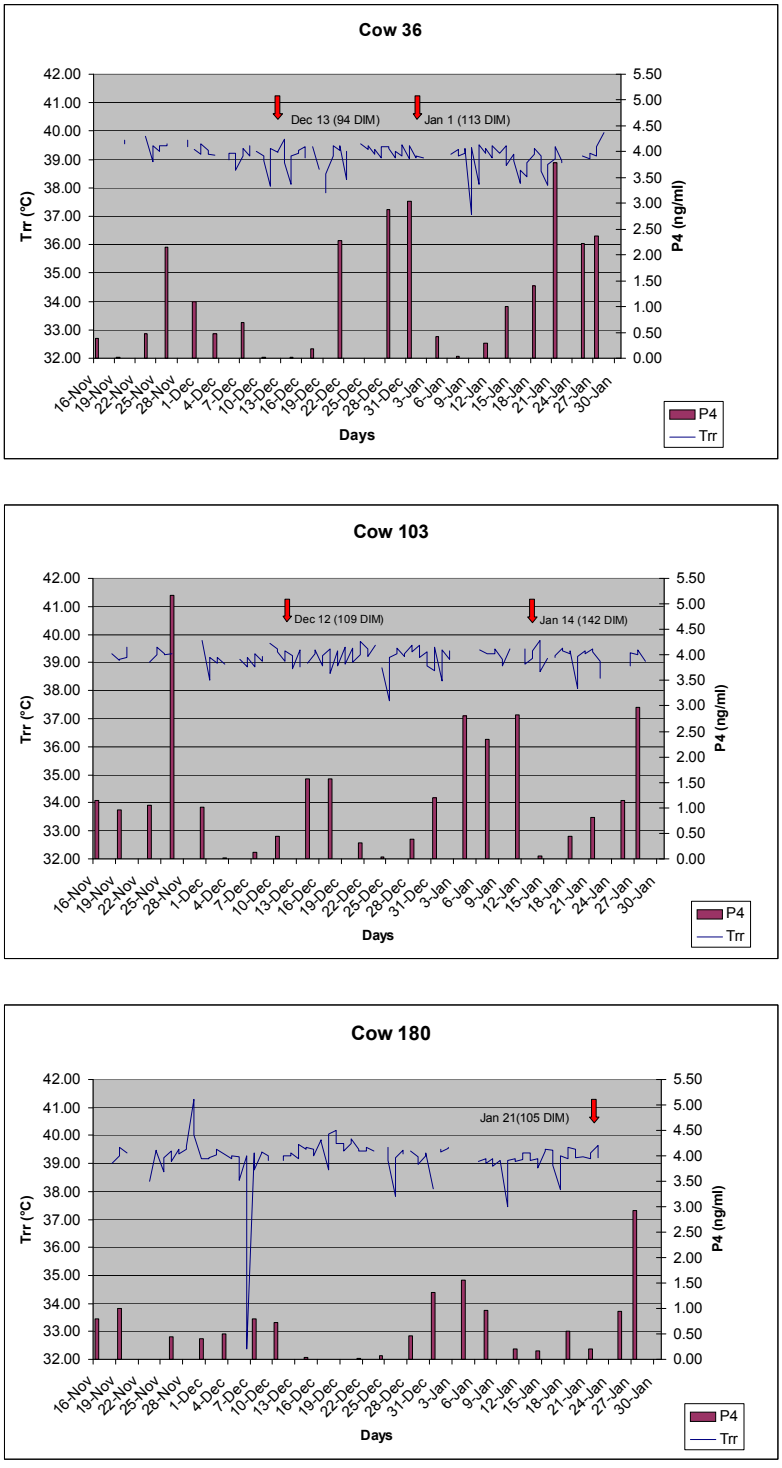


Figure 8. Representative data for individual cows in Trial 2 showing Trr and P4 for cows that were subjected to AI but confirmed pregnant post trial. Red arrows represent AI dates without confirmed conception.

CHAPTER 5. Discussion

Trial 1 showed that twice daily monitoring of Tv was able to detect ovulation in a dairy cows, with a sensitivity of 82% when using a 4d baseline and a 0.3ºC deviation in Tv. Similarly, Redden et al. (1992) reported the same criteria to be ideal when detecting estrus using Tv monitoring every four minutes for 66 days using radiotelemetry Tv monitoring, and also used P_4 has their golden standard. They reported the PPV for their system to be 82%. The increased amount of observations per animal could attribute to why they obtained a higher PPV. Their system encountered some dropped data, and there was one occurrence of the transmitter being used being expelled from the cow. One case may not seem large, but with only 13 cows on trial that would lead to a rate of 8%, which could cause problems when using a larger group. It is also unknown how having a transmitter anchored in the vagina could affect conception rates. Although the PPV for our study was not ideal at 50%, the NPV was high, at 95%. This is important because it indicates that, when using twice-daily monitoring of Tv, there is a 95% certainty that the indications of no ovulations are true, which is just as important on farm. This means that while there is a 50% that you are correctly identifying ovulation as present, there is 5% chance of saying there is no ovulation when one has occurred. This, combined with twice-daily monitoring of Tv having substantial agreement to P_4 monitoring of ovulation, justified the follow up trial done with the CTMS.

A previous study done using the CTMS reported a MR of 93% for the CTMS when they were set up so beef heifers had to pass through the panels to access water (Small et.al. 2008), while the current study only had a MR of 47%. The previous study had an average

of seven readings a day per animal. In this study we only took acquisitions twice a day, but based upon the position of the panels we had expected to see a similar MR. With the panels set up at the end of the milking parlour, it was ensured that each cow would pass through the panels twice a day so we were able to predict the exact amount of readings each animal would have, so therefore out expected and actual results should have been identical, under ideal conditions.

Pedometer systems vary in their PPV for estrus detection. The system used by Maatje et al. (1997) had a PPV of 66% while Roelofs et al. (2005a) reported it to be 95%, both using lactating dairy cows, and Kyle et al. (1998) one of 85.7% while studying beef cows. The majority of researchers reported a range between 70 – 83%, with an average of 76% (Koelsch et al., 1994; Maatje et al., 1997; Roelofs et al., 2005a; Løvendahl and Chagunda 2010), while in our study the CTMS had one of 50%. Many of these pedometer systems were radiotelemetry, which has similar technology to the panel system. Although the difference in PPV may seem substantial, the amount of dropped data due to the malfunctions in the panel system would cause this difference to be skewed, not allowing for a proper comparison of the two systems.

Radiotelemetry monitoring using pressure sensors had a detection efficiency (calculated based on number of cows detected in estrus divided by the number of cows synchronized to enter estrus) average of 91.7% and visual observation one of 98% in lactating dairy cows (Xu et al., 1998; Cavalieri et al., 2003). Our study found visual observation to have a PPV of 85%. This high PPV for visual observation is the reason that it is one of the

most commonly used methods for on farm estrus detection, usually in combination with another technique. The same can be said for radiotelemetry, since pressure pads were used to detect mounting behaviour, which is related to visual observation of estrus behaviour. There does not necessarily need to be a comparison between visual detection and the CTMS, since the system is not intended to replace visual observation but be used in conjunction with it.

Peralta et al. (2005) did a comparison of Heat Watch (a pressure activated wireless system used to detect mounting behaviour), pedometer activity and visual observation techniques to detect estrus in a herd of lactating Holstein cows. Visual observation had a MR (calculated by dividing the number of estrus periods detected by all and each of the systems by the total theoretical number of possible estrus periods (n=570) for all cows) of 49%, Heat Watch of 48% and an activity sensor system had one of 37%. When the three techniques were all combined to detect estrus there was a MR of 80%. The PPVs reported by Peralta et al. (2008) for the individual techniques seem on the low side compared to other studies, but, especially when discussing visual observation, was more true to what happens on farm. Instead of constant observation, there was a twice-daily visual observation period and another one during the night milking. The study also showed that combining techniques to monitor estrus will result in higher a MR. A MR of 80% is good, but it shows that there is still room for improvement that, when functioning, the panel system could provide, since it was able to achieve a PPV of 50% when the MR was only 47%. Peralta et al. (2005) also did not mention any false indications of estrus,

and there was no mention of whether this was because there were none or if they were not taken into account.

Other studies in which temperature was monitored as an indicator of estrus had PPVs of 82% and 85.7% (Redden et al., 1993; Kyle et al., 1998). The panel system had a PPV of 50%. Although at first glance this may be discouraging, it must be taken into account that the CTMS didn't have as many opportunities to detect positive ovulations due to the fact that some estrous cycles were missed because of the gaps in the data.

It is interesting to note that, despite the loss of data, both Trial 1 and Trial 2 had similar results for the best criteria for ovulation detection. In both, a temperature variation of 0.3ºC was best. Trial 1 found that using a 4-day baseline was ideal, while Trial 2 found a 5-day baseline to be more beneficial. This shows at least some validity in the CTMS, since it was able to achieve similar results as the manual Tv measurements. Another study done by Kyle et al. (1998) reported that, in Hereford-cross beef cattle, a 0.4°C change in Tv and a 2d or 3d baseline was the best criteria for detecting ovulation and that this 0.4°C change should occur for at least three hours. It is possible that the cause of the slight difference in criteria was that Kyle et al. (1998) took measurements every four minutes and then averaged temperatures for every hour, while in the present study Trr readings were only obtained twice a day. While hourly temperature readings may give a more accurate time of estrus, it is not always practical on a farm. Kyle et al. (1998) used Tv measurements as opposed to Trr, so that may also account for the difference, though more research would need to be done to be certain. Redden et al. (1993) reported that the

criteria of a 0.3ºC deviation over a 4d baseline increased their PPV for determining estrus. These results are similar to what was found in this study, providing confirmation that the panel system has potential as a detector of ovulation.

There may be some other causes for missed ovulations besides dropped data. Reticulo-rumen temperatures were taken while leaving the milking parlour because it was assumed that by the time the cattle made it from the holding pen and through the parlour, a decrease in Trr caused by drinking water before milking would not be evident. Preliminary studies done since this study and a study done by Bewley et al. (2008b) showed that a large drink of cold water can cause a decrease in Trr for as long as three hours, if not more. One cow in particular, Cow 37, showed large decreases in Trr at multiple times during the study that may have been caused by drinking. In turn, these decreases could have masked peaks in Trr caused by ovulation. Another limiting factor was that, when the trial was started and boluses were administered, the boluses were distributed to the cows that had most recently calved. In some cases, that was enough DIM for them to have already been bred and, in some cases, have conceived. Boluses should have been administered as cows calved and were put back into the production line so that the entire post-partum period was being monitored. Because 29% of animals were pregnant for the whole trial and 17% were pregnant for part of the trial, it decreased the number of possible ovulations to detect, thus narrowing the already small data pool.

Another potential limiting factor of the current study was the use of P_4 as the standard to detect ovulation. In most studies, levels above 1 ng/ml in blood indicate a fully

developed, functioning CL (Battocchio et al. 1999; Redden et al., 1993). While this threshold appeared appropriate for the first trial, in the second trial it was noted that overall the P_4 levels were rather low. Typically in the literature, 1ng/ml is used as a threshold in studies using blood and whole milk samples (Battocchio et al. 1999; Peterson et al. 2008). The samples for this trial were skimmed due to fat separating from the samples during the procedure. There has been very little research done with skim milk samples, and since P_4 is a fat-soluble hormone we decided to use 0.5 ng/ml as the threshold point for this study. It would be expected that skim milk would have less P_4 than whole milk samples (Colazo et al., 2008). This may have caused some points to be considered ovulations when they were not, thus causing the panels to classify points as FN when they were actually TN. The overall range for milk P_4 was lower for trial 1 than trial 2, with the high for trial 1 being 4.33 ng/mL for trial 1 and 8.79 ng/mL for trial 2. This may have been due to the additional thawing of the samples before analysis that samples from trial 1 were subjected to. The samples were thawed at 55ºC to be separated into aliquots and stored. Eissa et al. (1995) reported that there will be a decrease in P_4 seen in samples thawed at 37ºC, so the high thaw temperature used in this trial could have lowered overall P_4 concentrations.

Although, in this study, the CTMS was not a good option for ovulation detection, it would be presumptuous to write its potential off completely. The CTMS did show effective non-invasive detection of Trr, finding an average Trr of 37.9ºC, which is very close to the expected Tb of a dairy cow, which is around 38.0ºC (Beatty et al. 2008). After the CTMS was changed from wireless transmission to hardwiring, the functionality

of the panels increased greatly and it was used successfully for another trial after this one was completed. In hindsight, the panels should have had a short testing period before the study began to ensure that everything was running smoothly. Since the CTMS is still a relatively new technology, technical support was limited. Technicians familiar with the panel system were not available for on-farm assistance after the system was installed, so when there were problems consultation could be lengthy. This led to the system being out of commission for days, sometimes weeks, at a time while problems were being resolved. For future studies, and for the system to be a practical option for farmers, technical support will have to be more timely and convenient.

CHAPTER 6. Conclusion

Twice daily monitoring of Tv was shown to be able to detect ovulation in the high performing, lactating dairy cow.

Monitoring of Trr by the CTMS may be useful as a supplemental aid for ovulation detection. The criterion of a 0.3ºC deviation from a 5-d baseline was selected as the best for the CTMS and was similar to the recommendations reported in other studies. The work showed that twice daily acquisitions of Tv shows proof of concept for detection of ovulation.

There were many complications and limitations in this project. Repeating this project with the panels hardwired from the beginning, administering boluses as cows calved and having technicians familiar with the system on hand would be beneficial to obtain a more accurate picture of their functionality.

In conclusion, the CTMS could not be confidently recommended as an on farm detection tool in its current operational condition. There is still much research that needs to be conducted with the CTMS and technical issues that need to be corrected before it is ready for consumer use.

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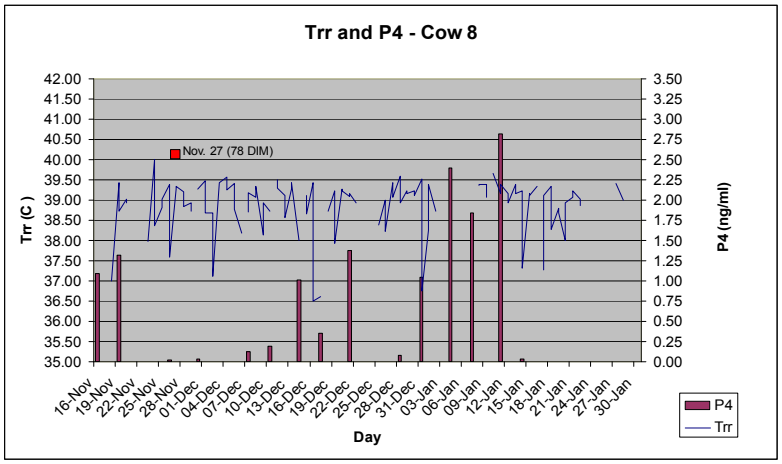
Appendix A. Raw data for Trial 1 cows

Cow Ear Tag	Barn #	Birth Date	Calving Date	AI Dates	Standing Heat	Comments	Preg Confirmed	Vaccine Date	Illness	Cull Date
30	666	01/18/2007	11/01/2009	03/28/209	06/03/2009		05/25/2009 135 DIM			
35	658	12/15/2006	22/01/2009	11/04/2009	03/20/2009	05/05/2009 - 3 teats		02/18/2009		08/17/2009
42	677	02/27/2007	26/01/2009	03/28/2010		12/28/2009 - Trim 01/13/2010 - Trim	05/25/2009 120 DIM			
62	670	02/02/2007	27/01/2009	04/38/2009 05/25/2009	03/13/2009	03/12/2009 - Trim 04/04/2009 - Ovsynch	07/23/2009 178 DIM	03/03/2009		08/20/2010 Sold
76	663	01/15/2007	30/01/2009	04/04/2009 05/11/2009 06/08/2009 07/05/2009		05/08/2009 - Trim	08/20/2009 203 DIM	03/04/2009		
78	678	02/28/2007	03/02/2009	01/05/2009		03/26/2009 - Ovsynch 05/07/2009 - Trim		03/03/2009		
79	665	01/17/2007	05/02/2009	04/29/2009 06/01/2009	04/27/2009	05/25/2009 - Trim	07/23/2009 168 DIM	03/17/2009		
80	676	02/25/2007	05/02/2009	04/18/2009	03/26/2009		05/25/2009 110 DIM	03/04/2009		11/25/2010 Died
111	669	01/30/2007	19/02/2009	05/23/2009 06/22/2009 07/10/2009 08/07/2009	04/18/2009		09/28/2009 222 DIM	05/17/2009		05/05/2010
116	673	02/14/2007	07/03/2009		03/19/2009	05/07/2009 - Trim		04/07/2009	04/27/2009 Metritis	
126	675	02/21/2007	08/03/2009	05/04/2009 05/25/2009	04/13/2009		07/23/2009 137 DIM			06/25/2010
129	688	04/29/2007	12/03/2009	05/20/2009	05/04/2009	05/08/2009 - Trim Lamanitis	07/23/2009 133 DIM	04/07/2009		
132	585	05/01/2006	19/03/2009	09/07/2009		05/08/2009 - Trim		04/07/2009		08/27/2009 Sold
134	681	10/04/2007	13/03/2009	06/14/2009		04/01/2009 - Trim	07/23/2009 132 DIM	04/07/2009		
155	2295	06/26/2005	21/03/2009	12/06/2009			07/23/2009 124 DIM	04/22/2009		02/15/2010 Died
167	671	04/02/2007	17/03/2009	05/23/2009 0 6/16/2009 07/10/2009	04/18/2009		08/20/2009 156 DIM	04/04/2009		
172	687	04/27/2007	19/03/2009	06/06/2009		05/07/2009 - Trim	07/23/2009 126 DIM	04/07/2009		
194	679	12/03/2007	23/03/2009	07/12/2009 07/27/2009 08/23/2009 08/28/2009 10/17/2009			11/30/2009 262 DIM	04/22/2009	03/31/2009 Retained Placenta (Pen)	
195	685	04/19/2007	27/03/2009		11/05/2009	04/04/2009 - Trim		04/22/2009		06/17/2009

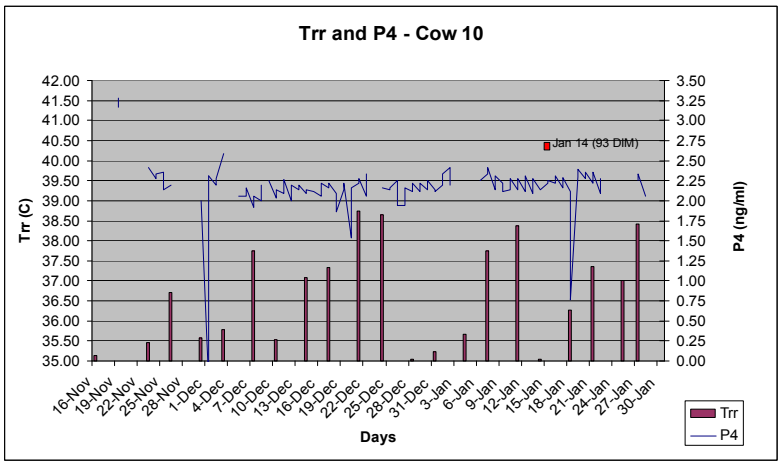
Appendix B. Raw data for Trial 2 cows

Cow Ear Tag	Bolus	Barn #	Birth Date	Calving Date	AI Date	Standing Heat	Comments	Preg Confirmed	Vaccine Date	Illness	Cull Date
3	3539	733	11/05/2007	12/10/2009		10/23/2009	Deceased				12/11/2009
8	4424	264	03/24/2002	09/10/2009	11/27/2009	10/13/09	01/12/2009 - Trim 01/18/2010 - Trim		11/23/2009	01/25/2010 Swollen Udder	01/04/2010
10	78246	545	10/21/2007	10/13/2009	01/14/2010	03/26/2010	09/05/2009 - Trim 01/13/2010 - Trim		11/23/2009		04/15/2010
16	3629	731	10/31/2007	10/14/2009	12/31/2009 01/28/2010	12/28/2009	09/04/2009 - Trim 12/29/2009 - Trim	03/29/2010 165 DIM	09/23/2009		
25	4404	618	05/22/2006	09/04/2009	11/20/2009	10/28/2009		12/28/2009 115 DIM	09/23/2009	10/17/2009 Mastitis	
27	4215	145	02/27/2004	10/04/2009	01/14/2010		10/07/2009 - Trim	03/29/2010 155 DIM	11/23/2009		
28	79343	712	09/08/2007	10/20/2009	01/01/2010	08/12/2009	11/05/2009 - Trim	02/22/2010 125 DIM	11/23/2009		
32	3890	144	02/14/2004	09/23/2009	12/12/2009 01/13/2010 01/23/2010 02/15/2010	11/13/2009		04/26/2010 216 DIM	10/16/2009		
35	81035	739	12/04/2007	11/03/2009	01/23/2010		12/29/2009 - Trim	03/29/2010 147 DIM	01/12/2009		
36	4004	611	05/08/2006	09/10/2009	12/13/2009 01/01/2010 02/02/2010 02/28/2010				10/16/2009		04/08/2010 Sold
37	77675	745	12/25/2007	11/15/2009	01/21/2010	01/17/2009		03/29/2010 160 DIM	01/12/2009		
39	79180		12/16/2007	11/15/2009	01/11/2010 02/03/2010	12/20/2009	11/06/2009 - Trim 01/18/2010 - Trim	03/09/2010 140 DIM	01/12/2009		
43	3876	343	03/07/2003	09/01/2009	10/29/09 11/21/09	09/09/2009	11/05/2009 - Trim	12/28/2009 118 DIM	09/23/2009	09/26/2009 Metritis	
48	3427	631	08/28/2006	08/27/2009	09/11/2009	10/18/2009	11/11/2009 - Trim	12/28/2009 123 DIM	09/23/2009		
54	3644	645	10/25/2006	09/12/2009	11/30/2009	06/11/2009	01/13/2009 - Trim	01/25/2010 135 DIM	10/16/2009		
55	3432	612	10/23/2006	08/25/2009	12/03/2009	01/10/2009	11/04/2009 - Trim 12/16/2009 - Trim 12/29/2009 - Trim 01/01/2010 - Open 01/02/2010 - Open				04/22/2010
58	3605	641	10/23/2006	09/20/2009	12/06/2009 12/31/2009 01/25/2010 02/18/2010 03/15/2010	11/13/2009	04/15/2010 - Trim	04/26/2010 213 DIM	10/16/2009		07/29/2010
60	3433	429	10/13/2004	08/17/2009	10/19/09		11/04/2009 - Trim 12/12/2009 - Trim	11/30/2009 105 DIM	12/06/2010		
73	3794	475	11/14/2006	10/07/2009	08/26/2009				12/06/2009		04/03/2010
75	4914	649	10/24/2006	09/26/2009	01/07/2010 02/04/2010		11/05/2009 - Trim		10/16/2009		
85	4733	643	10/24/2006	10/12/2009	12/19/2009		01/13/2010 - Trim 02/18/2010 - Abort	01/25/2010 93 DIM	11/23/2009	10/18/2009 Metritis (Pen)	
103	4479	103	06/30/2007	08/25/2009	12/12/2009 01/14/2010 02/06/2010 03/12/2010	11/11/2009			09/23/2009		04/08/2010 Sold
106	4198	705	06/30/2007	08/26/2009	11/05/2009 11/26/2009		11/04/2009 - Trim	01/25/2010 152 DIM	10/16/2009		
109	3664	725	10/08/2007	09/01/2009	11/11/2009	07/10/2009	09/15/2009 - Trim	12/28/2009 118 DIM	09/23/2009		
120	77695	392	10/12/2003	11/17/2009	02/18/2010 04/01/2010	12/31/2009	01/01/2010 - Trim 04/03/2010 - Trim		01/12/2009		06/05/2010
140	3570	726	10/08/2006	09/10/2009	11/18/2009		11/04/2010 - Trim	12/28/2009 127 DIM	10/26/2009		
141	8135	721	09/16/2007	09/13/2009	12/03/2009	10/18/2009		01/25/2010 136 DIM	10/16/2009	Abort	
144	3600	433	03/22/2004	08/29/2009	12/21/2009 02/05/2010			03/29/2010 213 DIM	09/23/2009		
153	3808	538	05/27/2007	09/17/2009	12/06/2009 01/28/2010 02/17/2010	11/14/2009		03/29/2010 194 DIM	10/16/2009		
158	4169	736	11/19/2007	09/20/2009	12/13/2009	10/23/2009	01/13/2010 - Trim	01/25/2010 129 DIM	10/16/2009		
159	3998	718	09/09/2007	09/26/2009	12/04/2009 01/14/2010	08/10/2009		02/22/2010 149 DIM	10/16/2009		

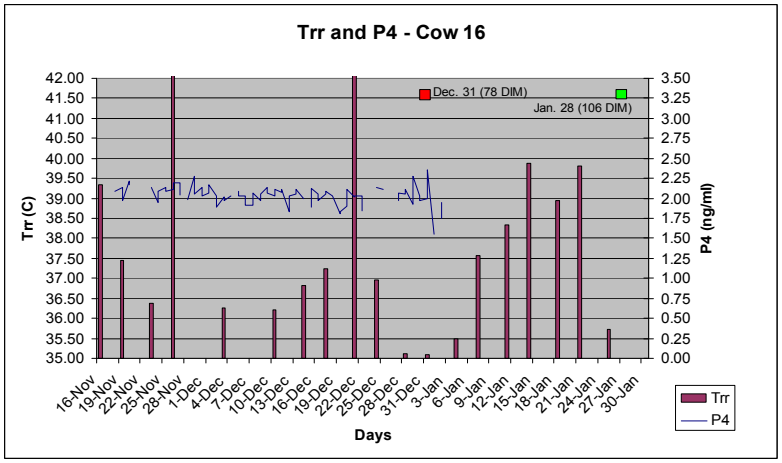
Appendix C. Cow 8



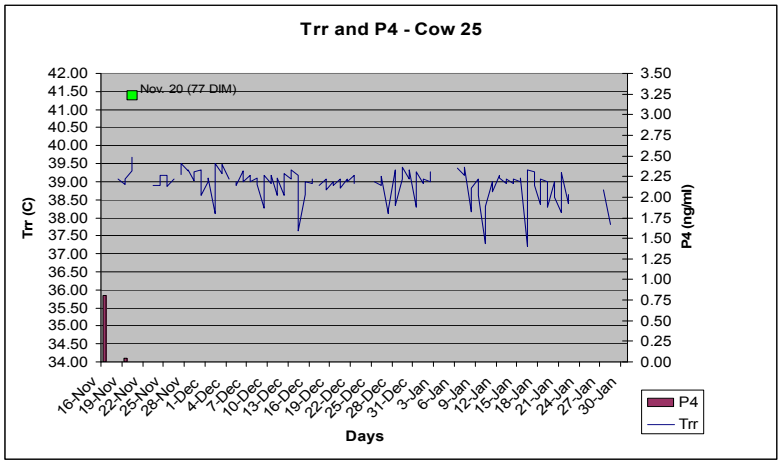
Appendix D. Cow 10



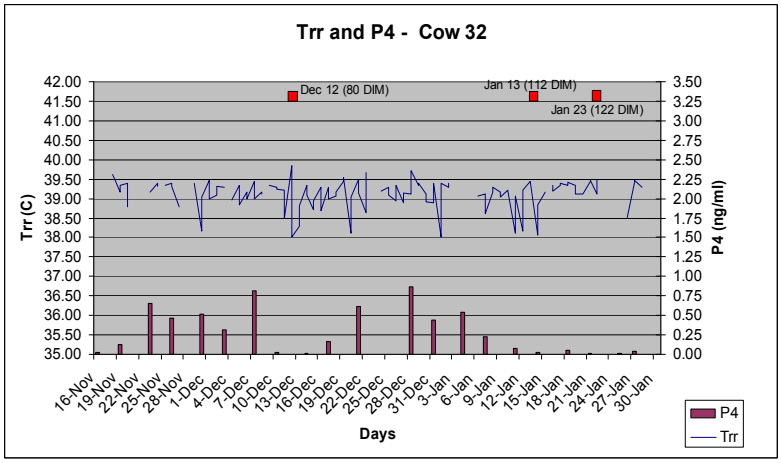
Appendix E. Cow 16



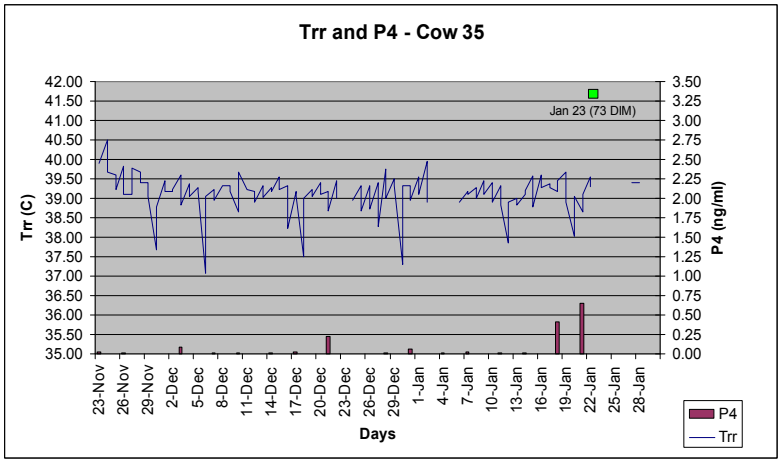
Appendix F. Cow 25



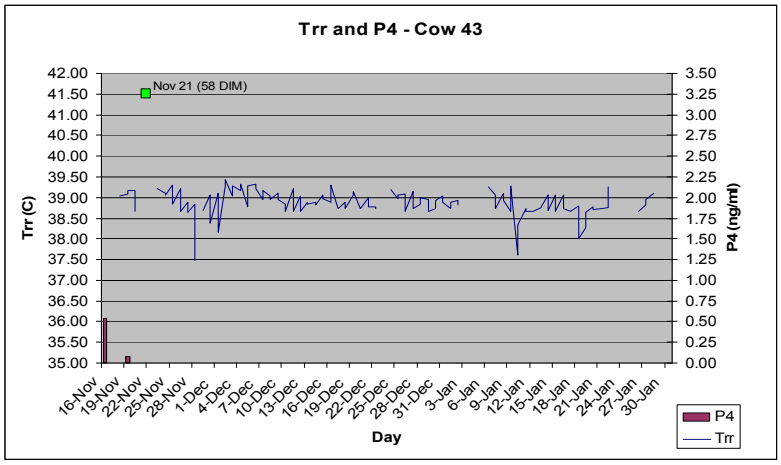
Appendix G. Cow 32



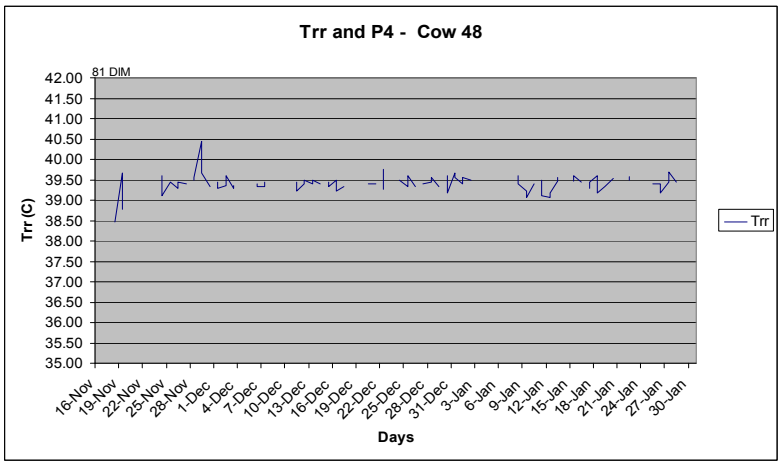
Appendix H. Cow 35



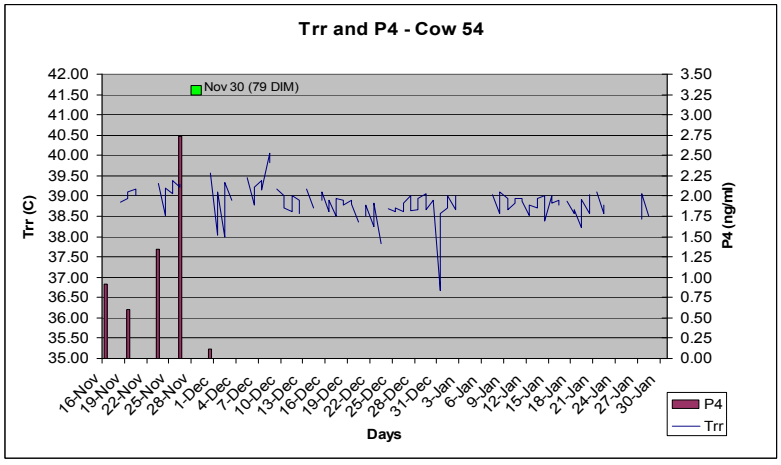
Appendix I. Cow 43



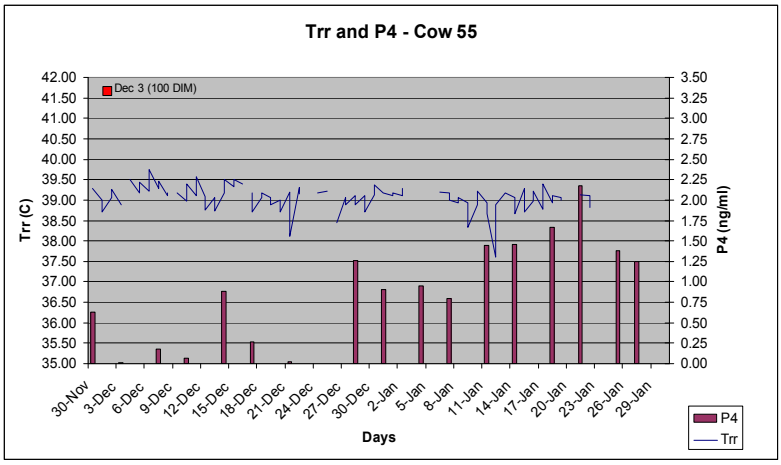
Appendix J. Cow 48



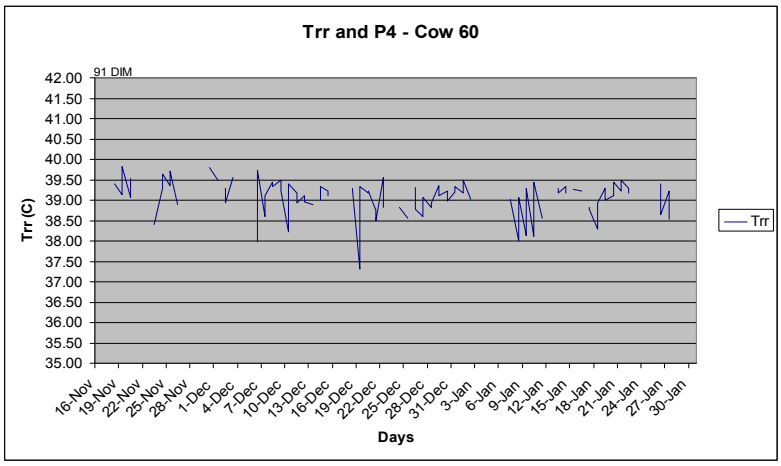
Appendix K. Cow 54



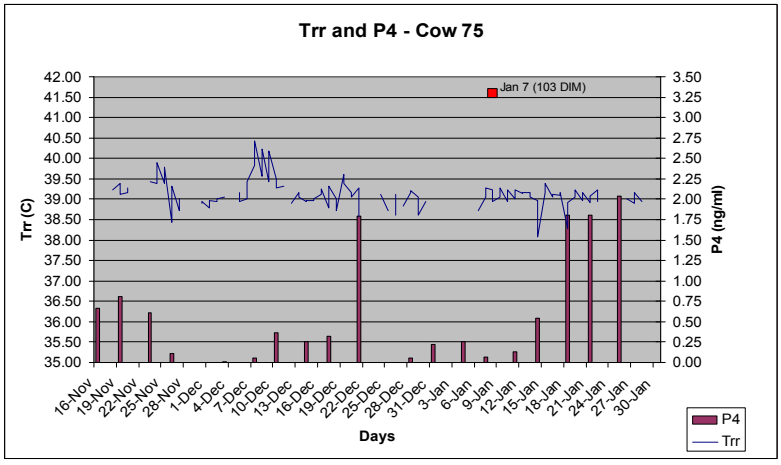
Appendix L. Cow 55



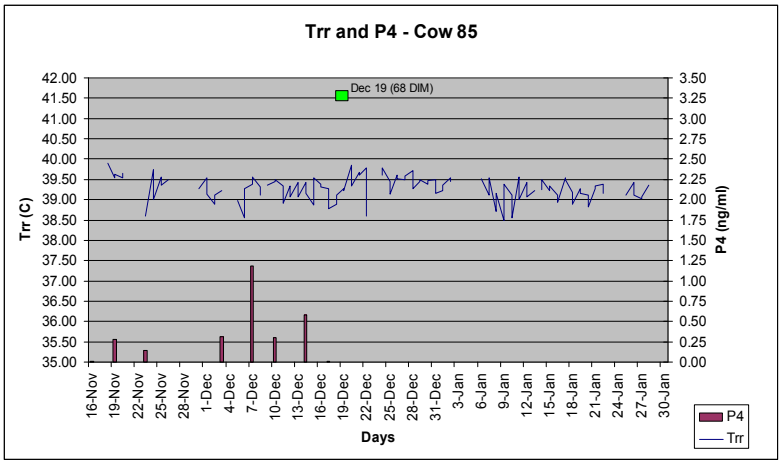
Appendix M. Cow 60



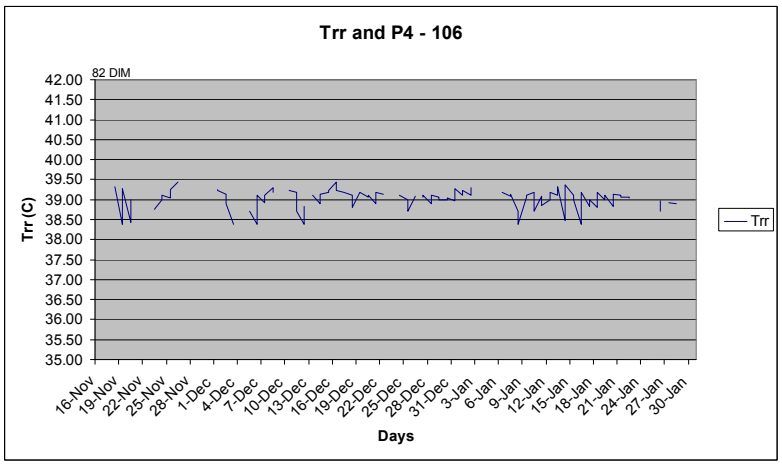
Appendix N. Cow 75



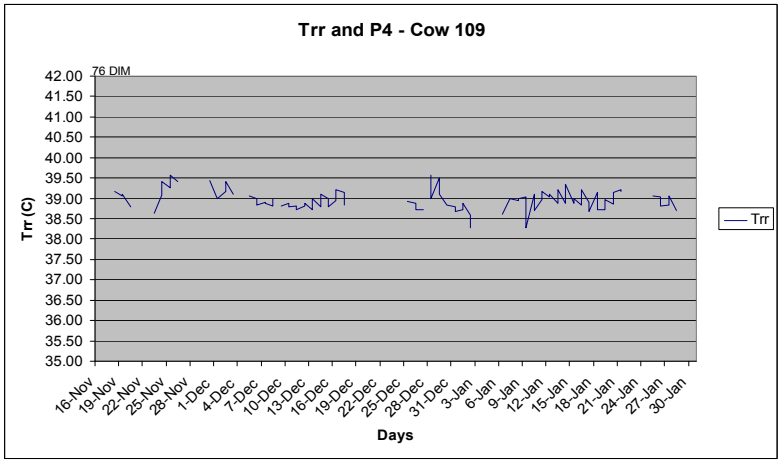
Appendix O. Cow 85



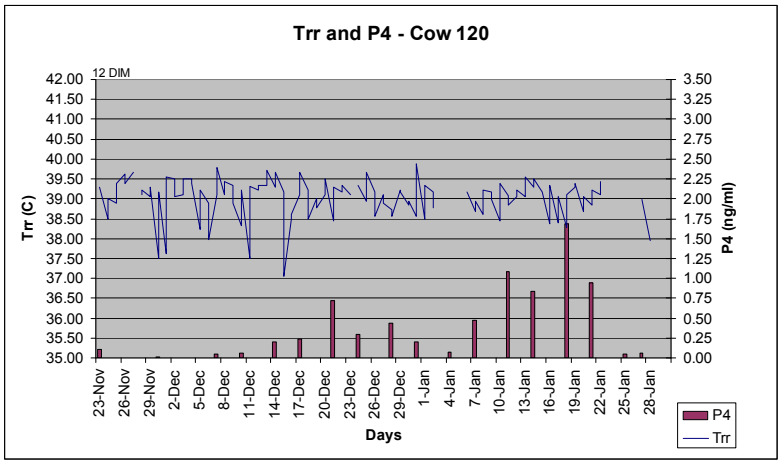
Appendix P. Cow 106



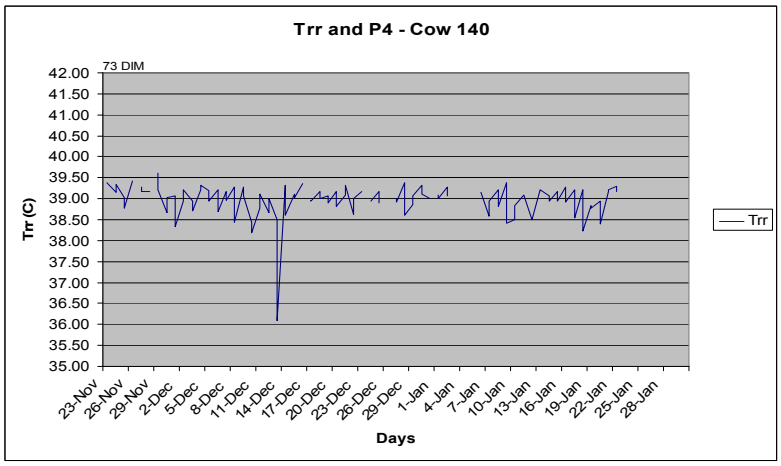
Appendix Q. Cow 109



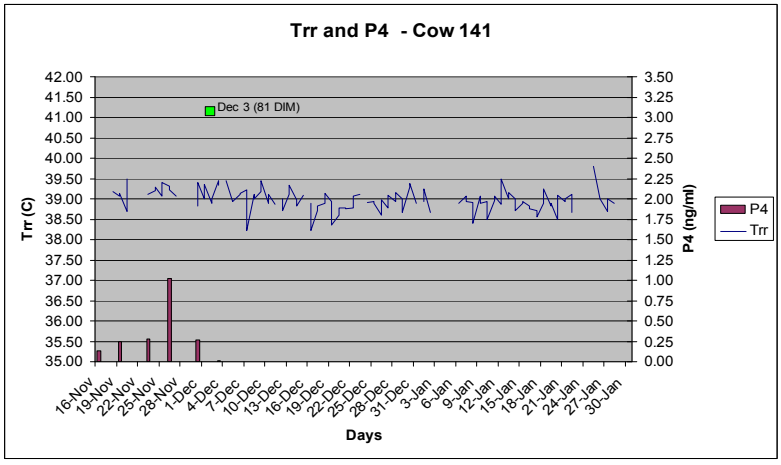
Appendix R. Cow 120



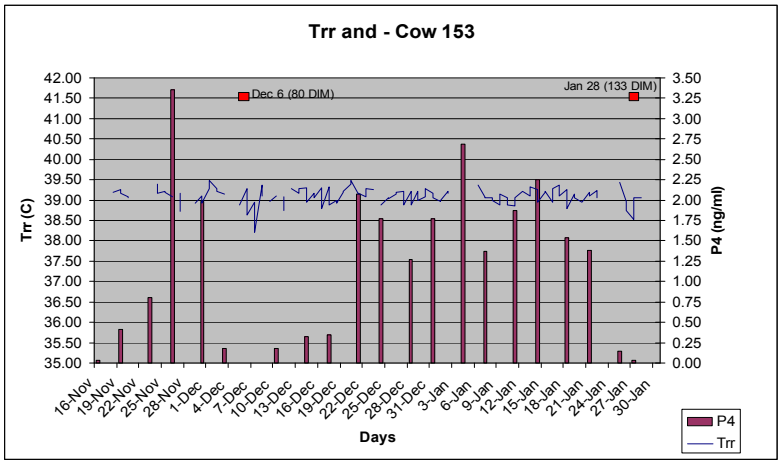
Appendix S. Cow 140



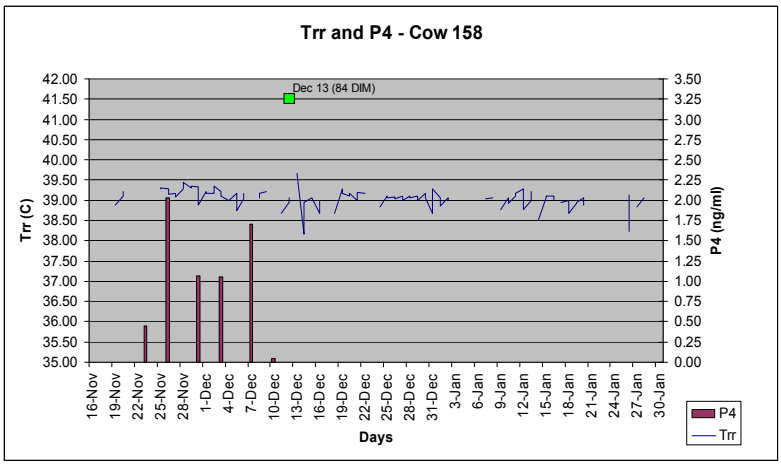
Appendix T. Cow 141



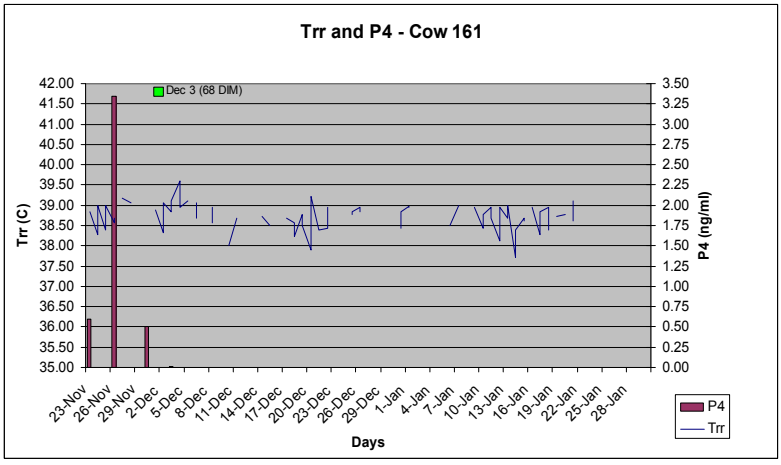
Appendix U. Cow 153



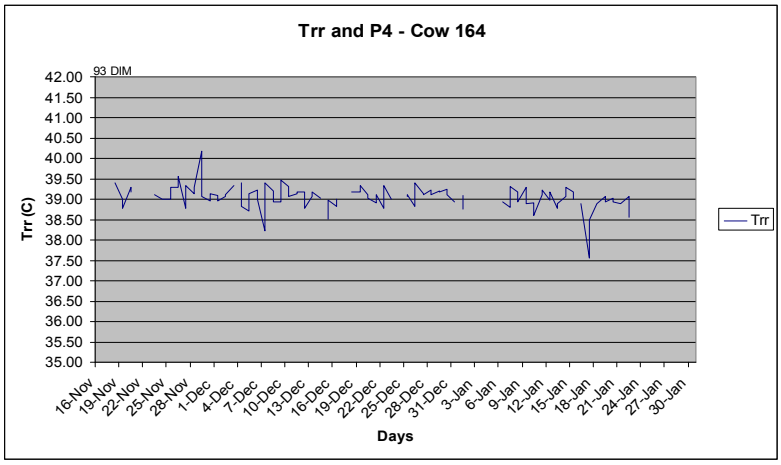
Appendix V. Cow 158



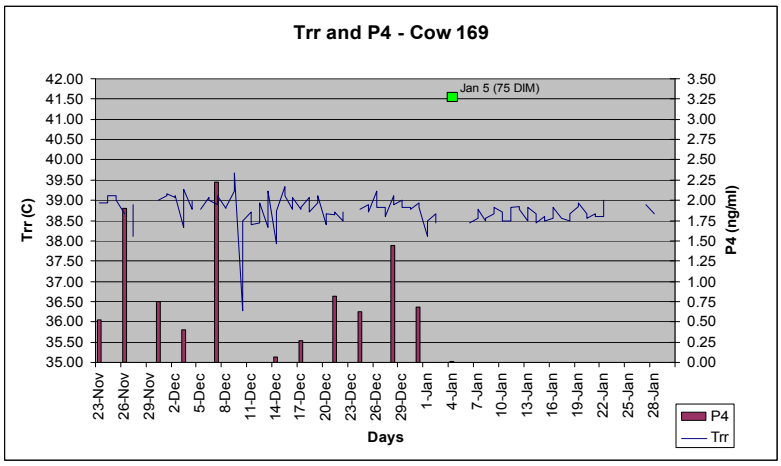
Appendix W. Cow 161



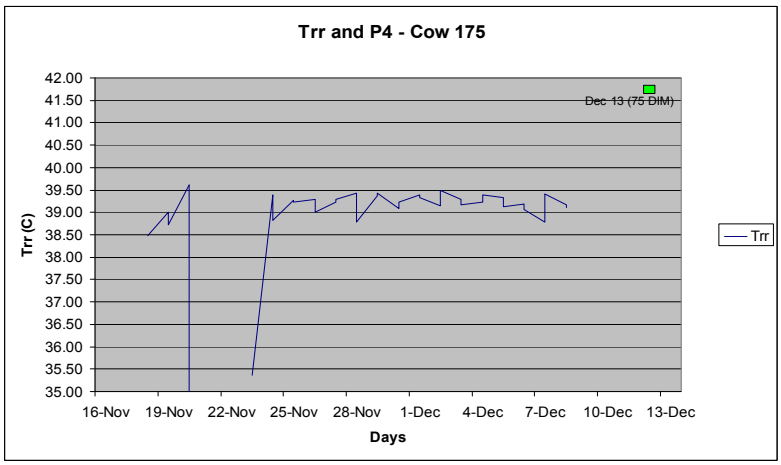
Appendix X. Cow 164



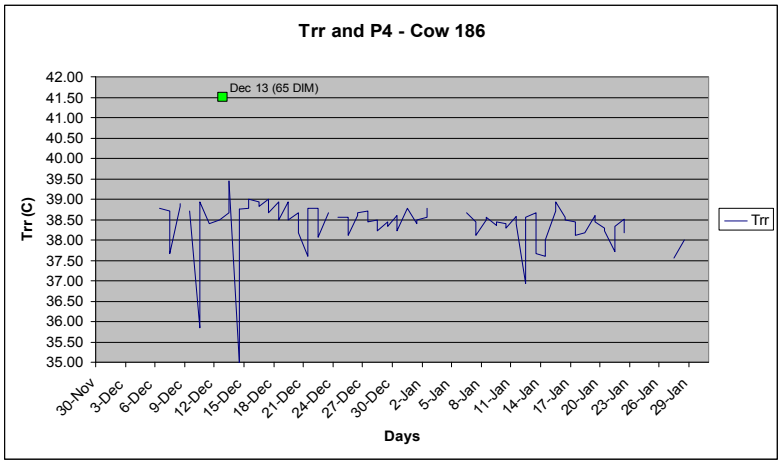
Appendix Y. Cow 169



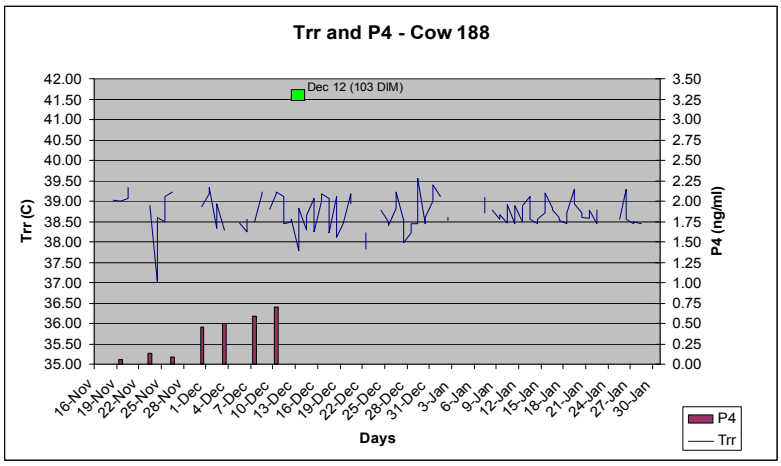
Appendix Z. Cow 175



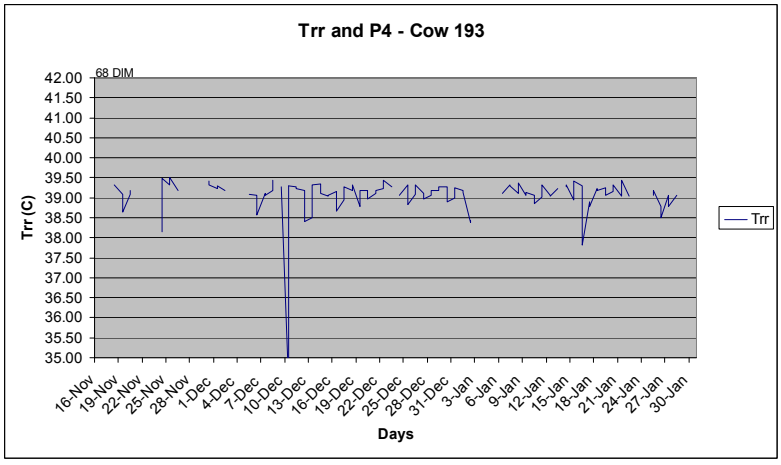
Appendix AA. Cow 186



Appendix BB. Cow 188



Appendix CC. Cow 193



Appendix DD. Cow 197