

PRE-PARTUM BEHAVIOUR AND INTAKE IDENTIFY DAIRY COWS  
AT RISK FOR POST-PARTUM METRITIS

by

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## ABSTRACT

Metritis is a disease of particular concern after calving due to its negative effects on the reproductive performance of dairy cows. Previous work has shown that cows at risk for post-partum metritis have lower feeding times in the days before calving. However, the authors did not monitor individual dry matter intake (DMI), a measure that may be more sensitive in the detection of illness, or other behavioural or intake measures such as drinking or social behaviour that may prove to be useful predictors of disease. The objective of this study was to determine which pre-partum measures are most sensitive in predicting post-partum metritis. Feeding and drinking measures were collected from 101 Holstein dairy cows from 2 wks before until 3 wks after calving using an electronic monitoring system. Social behaviour at the feed bunk was assessed from video recordings. Metritis severity was diagnosed based on daily rectal body temperature and vaginal discharge that was assessed every 3 d after calving until d+21. In this study, 12% of cows were classified as severely metritic and 27% as mildly metritic. Feeding time and DMI were best able to identify cows at risk for metritis. Cows that developed severe metritis spent less time feeding and consumed less feed relative to healthy cows 2 wks before any observation of clinical signs of infection. For every 10-min decrease in average daily feeding time during the wk before calving the odds of severe metritis increased by 1.72, and for every 1 kg decrease in DMI during this period, cows were nearly 3 times more likely to be diagnosed with this disorder. During the wk before calving cows that were later diagnosed with severe metritis had lower DMI and feeding times during the hours following fresh feed delivery. During this period these cows also engaged in fewer aggressive interactions at the feed bins compared to cows that remained healthy. This research is the first to show that social behaviour may play an important role in transition cow health. Further research is required to determine how management practices should be changed to reduce illness in transition dairy cows.

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## LIST OF ABBREVIATIONS

**ADF:** Acid detergent fiber

**BCS:** Body condition score

**BHBA:** Beta-hydroxybutyrate

**BT:** Body temperature

**BW:** Body weight

**CP:** Crude protein

**d:** Day

**DM:** Dry matter

**DMI:** Dry matter intake

**h:** Hour

**HPA:** Hypothalamic-pituitary-adrenal

**min:** Minutes

**mo:** Month

**NDF:** Neutral detergent fiber

**NEFA:** Non-esterified fatty acid

**NE<sub>L</sub>:** Net energy of lactation

**RFID:** Radio frequency identification

**SD:** Standard deviation

**SE:** Standard error

**TMR:** Total mixed ration

**VD:** Vaginal discharge

**wk(s):** Week(s)

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## **CO-AUTHORSHIP STATEMENT**

This study was designed collaboratively by: Juliana Huzzey and Drs. Marina von Keyserlingk, Dan Weary and Doug Veira. Juliana was responsible for setting up and managing the experiment at the UBC Dairy Education Centre. Juliana analyzed the data and prepared the manuscript under the guidance of her supervisors, Drs. von Keyserlingk, Weary and Veira.

# CHAPTER 1: INTRODUCTION

## THE CANADIAN DAIRY INDUSTRY

The dairy industry is Canada's 4<sup>th</sup> largest agriculture sector (CDIC, 2006). The majority of dairy farms in Canada are located in Ontario and Quebec (81%), followed by 13% in Western Canada and 6% in the Atlantic Provinces. In 2005, the average herd size was 66 cows (CDIC, 2006). In 2005 there were approximately 1,048,600 dairy cows on 16,224 farms (Statistics Canada, 2006). Farm cash receipts for the Canadian dairy sector totaled 4.6 billion dollars (CDIC, 2006) making this an important industry in the Canadian economy.

The income that a dairy cow generates comes from her milk production, sale of surplus offspring and finally, her carcass value (Gröhn et al., 2003). Obviously factors that influence each of these income streams (e.g. cattle genetics, nutrition, environmental management, and herd health) can affect the overall profitability of a dairy herd. Perhaps one of the most challenging areas for both producers and scientists has been addressing the issues of herd health.

Health disorders can generate both direct disease related costs (drug treatment, veterinary costs and death of animals) as well as indirect costs. Diseases can influence production efficiency by decreasing milk production (Deluyker et al., 1991; Rajala and Gröhn, 1998), reducing reproductive efficiency and increasing the risk of involuntary culling (this occurs when the farmer is forced to remove a productive, profitable cow due to illness, injury, infertility, or death) (Gröhn et al., 2003). An example is provided by LeBlanc et al. (2002a) who reported that cows with clinical endometritis (a uterine

infection) took 27% longer to become pregnant and were 1.7 times more likely to be culled for reproductive failure than cows without endometritis.

## THE TRANSITION COW

A major challenge for dairy producers and veterinarians is to maintain healthy dairy cows throughout the transition period. The transition period (also referred to as the periparturient period) is typically defined as 3 wks before until 3 wks after calving (Grummer, 1995; Drackley, 1999). During the transition period there are many physiological, metabolic and endocrine challenges related to parturition and the onset of lactation (Bell, 1995). Nutrient demands of the dairy cow increase during this period to support the final stages of fetal development and, after calving, milk production. Bell (1995) reported that 4 days after calving the mammary gland utilized 97% of the energy consumed from the diet, meaning that there was insufficient energy available for maintenance. A further challenge is that dry matter intake (DMI) during the transition period is generally insufficient to meet the energy requirements for lactation and maintenance (Drackley, 1999).

Dairy cows are also forced to adapt to numerous management challenges during the transition period. On typical North American dairy farms the transition from pregnancy to lactation is marked by several social regroupings and changes in diet. The first group change, occurring approximately 3 wks before the cow's expected calving date, allows cows to be fed a diet with increased energy and nutrients to support the final stages of fetal development, and prepare the cow for the physiological and metabolic adaptations necessary for parturition and the onset of lactation (Overton and Waldron, 2004). This regrouping also enables producers to closely monitor the cows as

they approach their expected calving date. There is evidence, however, that regrouping has negative consequences on both behaviour and production. Phillips and Rind (2001) reported that regrouped animals had shorter feeding times, longer standing times and decreased milk production relative to cows kept in a stable group. When new cows are introduced to a pen the group dynamics change and this can lead to increased levels of aggression among individuals as social relationships become established (Tennessen et al., 1985).

As parturition approaches cows are moved again, this time to a maternity pen where they are usually isolated from the herd. Social isolation in unfamiliar surroundings has been shown to elicit stress responses in dairy cows in the form of increased heart rate, high cortisol concentrations, and increased vocalizations (Rushen et al., 1999). After calving the calf is removed and the cow is moved again. This time she joins the lactating herd and is fed a new diet formulated to provide more energy to support the increased nutrient demands for lactation (NRC, 2001).

These challenges likely contribute to the high incidence of metabolic and infectious diseases observed in dairy cows after calving. Studies on improving health during the transition period have focused on the role of nutrition on metabolic and physiological processes. For example, nutritional deficiencies (low dietary selenium and Vitamin E) have been shown to impair the immune system and have also been associated with a high incidence of mastitis and retained placenta (Hogan et al., 1990; Weiss et al., 1990; LeBlanc et al., 2002b). In addition, reduced immune function before calving has been associated with negative energy balance, low DMI and an increased susceptibility to uterine infection after calving (Hammon et al., 2006). Researchers have examined the effects of feeding energy dense diets during the pre-partum period for long and short durations (e.g. 21 or 60 d treatments); however, the effects of these

dietary treatments on health have been inconsistent (Mashek and Beede, 2001; Contreras et al., 2004). There has also been interest in determining how feed additives can be used to improve energy status and consequently health during the transition period. For example, providing dairy cows with a controlled release capsule of the ionophore, monensin, before calving reduced the incidence of energy-associated disease by 30% (Duffield et al., 2002). These studies have contributed to our understanding of how nutrition relates to health status; however, the incidence of disease around calving remains high.

In a review of 25 studies related to transition cow health, Ingvarsten et al. (2003) summarized the incidence of common production diseases (Table 1.1) and noted large variation in disease among herds. This variation in incidence is likely a consequence of different herd management practices and diagnostic criteria. Additionally, the incidence reported is from clinical diagnosis; however, many diseases occur sub-clinically (Ingvarsten, 2006). Incidence measures also depend on the quality and accuracy of the farms herd health records. Therefore, it seems likely that the actual percentage of dairy cows developing illnesses after calving is higher than current studies report.

The high incidence of disease during the transition period suggests the need for a new research approach to improve health management. Particularly promising is new work showing that changes in behaviour can be used to identify animals that are ill or at risk for disease (Sowell et al. 1998,1999; Quimby et al., 2001; Urton et al., 2005).

## BEHAVIOUR AND DISEASE

Animals that are acutely sick from a systemic infection commonly display a variety of symptoms including changes in body temperature, lethargy and decreased

appetite (Hart, 1988). For veterinarians these symptoms, particularly behaviours, have been considered to be a consequence of a debilitated physiological state that prevents the animal from engaging in normal behaviour (Aubert, 1999). However, Hart (1988) argued that sickness behaviour is a coordinated set of physiological and behavioural changes that develop in response to an infection. He proposed that fever, and the corresponding depressions in appetite and activity, are the body's adaptive responses for fighting the invading pathogen. An elevated body temperature occurs in response to an infection and improves immune function by increasing bacterial killing by neutrophils (Sebag et al., 1977) and by creating an inhospitable environment for bacteria to grow (Small et al., 1986). Behaviours that conserve energy and minimize heat loss (e.g. inactivity and decreased intakes) are also beneficial. For example, inactivity results in reduced muscular activity consequently causing the body to conserve energy for the increased metabolic costs of mounting a fever response. Additionally, the act of feeding can require movement to the feeding area, competition with others for access to feed, and the ingestion and digestion of food, all of which increase energy expenditure (Hart, 1988).

Differences in the behaviour of healthy and sick animals have been observed in feedlot steers and dairy cows. Using an electronic monitoring system, Sowell et al. (1998) found that healthy feedlot steers spent 30% more time at the feed bunk than morbid steers, and a higher percentage of healthy steers visited the feed bunk immediately following feed delivery. In a follow up study, Sowell et al. (1999) reported that healthy steers had more feeding bouts than morbid steers. Quimby et al. (2001), using the same electronic monitoring system, was able to detect animal morbidity approximately 4.1 d earlier than conventional methods typically employed in commercial feedlots (i.e. visual observation of clinical symptoms).

In a study with transition dairy cows, Urton et al. (2005) electronically monitored the feeding behaviour of 26 cows from 2 wks before until 3 wks after calving. Following calving, cows were diagnosed with either metritis or acute metritis. A retrospective analysis of the feeding behaviour between healthy and metritic cows revealed that not only was feeding behaviour different during the period of uterine infection, cows that developed acute metritis had lower feeding times, relative to healthy cows, beginning 12 days before clinical signs of infection.

These results indicate that behaviour can be used to identify disease days before a stockperson or veterinarian would otherwise diagnose illness. Recovery is faster when medical treatment is initiated early in the disease process. Milner et al. (1997) reported that early identification and treatment of mastitis, using milk conductivity tests, limited the severity of the disease by preventing clinical signs of infection from developing and resulted in less depression in milk yield. Consequently, monitoring feeding behaviour during the transition period may be a tool producers can use to identify and treat sickness sooner thus improving animal health.

In the studies by Quimby et al. (2001) and Urton et al. (2005) it is unclear whether the reduction in feeding activity in cattle that were later identified as sick was due to an expression of sickness behaviour or whether the difference in behaviour contributed to the development of the disease. Changes in feeding behaviour may influence changes in intake, but how this occurs is not well understood. As discussed previously inadequate nutrients (Weiss et al., 1990) and intake (Hammon et al., 2006) before calving have been associated with an increased incidence of disease after calving. However, Quimby et al. (2001) and Urton et al. (2005) were not able to measure individual DMI. To date no work has examined the relationship between feeding time and DMI in individual transition dairy cows.

Cows that are less successful at gaining access to resources such as feed may be more susceptible to disease due to the nutritional deficiencies associated with reductions in intake. Work completed by Huzzey et al. (2006) showed that low-ranking cows at the feed bunk (i.e. cows that were displaced more than they displaced others) had lower feeding times in response to increased competition for feed relative to higher ranking cows (i.e. cows that displaced others more than they were displaced). To date, no research has explored the relationship between social behaviour, intake and health during the transition period. Monitoring social behaviour during the transition period may be another tool producers can use to identify cows that may have difficulty in gaining access to the feed and, consequently, are at greater risk of developing health complications.

## RESEARCH HYPOTHESIS

The aim of my research was to test the prediction that behavioural patterns (including feeding, drinking and social behaviour) would be different in healthy and sick cows during the transition period. Specifically, I hypothesized that cows with reduced feeding and drinking activity (depressions in intake and duration of feeding/drinking) during the wks before calving would be more likely to be diagnosed with metritis after calving. In addition, I predicted that these animals would be less successful at competitive interactions at the feed bunk during the period before calving relative to cows that remained healthy.

<b>Disorder</b>	<b>Mean (%)</b>	<b>Range (%)</b>
Milk Fever	4.6	0.2 – 8.9
Displaced abomasum	2.1	0.6 – 6.3
Ketosis	4.1	0.2 – 10
Retained placenta	7.8	3.1 – 13
Metritis	10.8	1.7 – 43.8
Mastitis	17.6	2.8 – 39

<sup>1</sup>Adapted from Ingvarsen et al. (2003)

**Table 1.1.** Mean and range for incidence of selected periparturient health disorders.<sup>1</sup>

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## **CHAPTER 2: PRE-PARTUM BEHAVIOUR AND INTAKE IDENTIFY COWS AT RISK FOR POST-PARTUM METRITIS**

### **INTRODUCTION**

There is much interest among dairy scientists in finding ways to improve the early identification of disease. Health disorders can have a major impact on the profitability of a dairy herd. As outlined by Gröhn et al. (2003), diseases can influence production efficiency in three ways: by reducing milk production, reducing reproductive performance, and by shortening the life expectancy of a dairy cow through increased culling rates. Early identification of sick cows is a critical component of any dairy herd health program.

During the transition period (typically defined as the period from 3 wks before until 3 wks after calving) dairy cows are vulnerable to metabolic and infectious disease; so early identification may be especially useful at this time. Metritis is of particular concern due to profound negative effects on the reproductive performance of dairy cows. Cows diagnosed with metritis experience longer calving to first service periods, longer periods from calving to conception, and increased failure to become pregnant (Borsberry and Dobson, 1989; LeBlanc et al., 2002; Gilbert et al., 2005). In their review, Gröhn et al. (2003) suggested that reproductive status of a cow was the single most important factor influencing culling decisions on farms, indicating that metritis likely contributes indirectly to the high rates of involuntary culling.

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A version of this chapter has been submitted for publication. Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. Pre-partum feeding behavior and intake identify cows at risk for post-partum metritis. *J. Dairy Sci.*

The incidence of metritis varies among studies from 8 to 53% (14.8%, Borsberry and Dobson, 1989; 7.6%, Gröhn et al., 1995; 53%, Gilbert et al., 2005; 16.9%, LeBlanc et al., 2002). This variation is likely due to differences in the methods used to classify uterine infections. The herd veterinarian is normally responsible for disease detection, but typically only during routine herd health checks; in many cases early warning signs of disease may go unnoticed. Thus practical methods for improved health monitoring would be useful.

Research on feedlot steers has shown that changes in feeding behaviour can be used to identify sick animals and even to predict morbidity. Sowell et al. (1998, 1999) reported that healthy steers spent longer at the feed bunk and had more feeding bouts than sick animals. A follow up study by Quimby et al. (2001) determined that electronic monitoring of bunk attendance by animals could be used to detect sick steers 4 d earlier than trained pen checkers. More recently, work by our research group has shown that reduced time at the feeder before calving can be used to identify dairy cows at risk for acute metritis (Urton et al., 2005). Unfortunately, dry matter intake (DMI) was not measured in any of these previous studies. Changes in intake must be mediated by changes in feeding behaviour, but how this occurs is not well understood. Previous work has assumed that those animals that spend more time at the feeder will consume greater amounts; however, animals may vary considerably in intake rates potentially weakening these relationships. Therefore one objective of this study was to explore the relationship between feed intake and feeding behaviour during the transition period, to determine which measures of intake and feeding behaviour are most sensitive for identifying cows at risk for post-partum metritis.

Water is considered to be one of the most important nutrients for a dairy cow (NRC, 2001). Studies have shown that water intake and feed intake are closely linked

(Murphy, 1992), and depressions in water intake result in depressions in feed intake (Burgos et al., 2001). Thus cows spending less time at the feed bunk may also spend less time at the water trough. Thus the second objective of this study was to determine if water intake or drinking behaviour are useful in identifying cows at risk for post-partum metritis.

Social behaviour may also provide an indication of a cows' susceptibility to disease. Increasing competition at the feed bunk results in lower feeding times among cows and increased aggressive interactions (Olofsson, 1999; DeVries et al., 2004), with socially subordinate cows being displaced most frequently, particularly at high stocking densities (Huzzey et al., 2006). Stress from aggression may negatively affect the immune system of a dairy cow during the transition period, a time when the cows' innate and acquired defense mechanisms are already at their lowest (Mallard et al., 1998). Further, social competition at the feed bunk during transition may limit a cows' ability to consume adequate DM. This could be particularly devastating to the dairy cow as her need for nutrients is high during the transition period in order to support fetal growth and lactation (Bell, 1995). Thus, the final objective of this study was to examine how social behaviour at the feed bunk (engaging or avoiding aggressive interactions at the feed bunk) during the pre-partum period was related to post-partum health status.

## MATERIALS AND METHODS

### Animals, Housing, and Diet

This study was conducted between August 2005 and March 2006 at the University of British Columbia's Dairy Education and Research Centre (Agassiz, BC,

Canada). All animals were cared for according to the guidelines of the Canadian Council on Animal Care (1993). During this 8-mo period a total of 32 primiparous and 69 multiparous (parity =  $2.28 \pm 1.32$ ; mean  $\pm$  SD) Holstein dairy cows were monitored. Parity was recorded at the beginning of the experiment. Animals were housed in pre-partum and post-partum group pens, each containing 20 freestalls fitted with a mattress (Pasture Mat, Promat Inc., Ontario, Canada) covered with 5 cm of sand bedding, 12 Insentec (Insentec, Marknesse, Holland) feed bins and 2 Insentec water troughs. Stocking density was maintained at 20 animals per pen. Group composition was dynamic with cows entering and leaving the experiment depending on their expected and actual calving dates. Cows entered the pre-partum pen  $25 \pm 2$  d before their expected calving date. They were moved to the maternity pen when they showed physical signs of imminent calving (i.e. udder enlargement, milk letdown, relaxation of tail ligament). The maternity pen consisted of a sand-bedded pack with 6 Insentec feed bins and 1 Insentec water trough. A maximum of 2 cows were kept in the maternity pen at any given time and cows were moved to the post-partum pen within 24 h after calving where they were monitored until 21 d post-partum. Cows in the post-partum pen were milked twice daily at approximately 0700 h and 1700 h.

Cows were fed twice daily at approximately 0800 h and 1600 h. Every wk, for the duration of the study, samples of total mixed ration (TMR) for both the pre- and post-partum groups were taken on Monday, Wednesday and Friday. The 3 samples were then pooled to create 1 representative weekly sample of the pre- and post-partum TMR. These samples were dried at  $60^{\circ}\text{C}$  for 2 d to determine DM. Dried weekly samples were then pooled into monthly samples and sent for nutrient analysis (Cumberland Valley Analytical Services INC., Maryland, USA) to determine the average ( $\pm$  SD) CP, ADF, NDF, and  $\text{NE}_L$  content of the feed over the 8-mo study. Cows in the

pre-partum pen as well as in the maternity pen were fed a close-up TMR consisting of 21.3% corn silage, 42.8% alfalfa hay, and 35.9% concentrate and mineral mix on a dry matter (DM) basis (DM:  $50.8 \pm 1.19$  %, CP:  $14.4 \pm 1.01$  % DM, ADF:  $35.0 \pm 2.74$  % DM, NDF:  $45.6 \pm 2.57$  % DM, and NE<sub>L</sub>: 1.40 Mcal/kg). Cows in the post-partum pens were fed a TMR consisting of 21.3 % grass silage, 14.7 % corn silage, 12.3 % alfalfa hay, and 51.7 % concentrate and mineral mix on a DM basis (DM:  $51.1 \pm 1.82$  %, CP:  $17.7 \pm 1.00$  % DM, ADF:  $23.7 \pm 1.42$  % DM, NDF:  $36.1 \pm 1.82$  % DM, and NE<sub>L</sub>: 1.66 Mcal/kg).

### Feeding Behaviour, Drinking Behaviour and Intake

An electronic feeding system (Insentec, Marknesse, Holland) was used to continuously monitor feeding and drinking behaviour as well as individual feed and water intakes for all experimental cows. Each cow had a unique passive transponder (High Performance ISO Half Duplex Electronic ID Tag, Allflex, Canada) attached to her ear tag. When a cow approached the bin an antenna detected the cows' transponder and the head gate opened allowing the cow access to feed or water. At the time the gate opened the Insentec system recorded the time and the initial weight in the bin. When a cow exited the bin, the head gate closed and the system again recorded the time and the weight in the bin. These data were used to record the duration of each visit to the bin and the amount of feed or water consumed. Daily DMI was determined by correcting daily as fed intakes for the DM content of the feed.

### Social Behaviour

Social interactions occurring at the feed bunk were monitored using video cameras (CCTV camera, model WV-BP330, Panasonic, Osaka, Japan) with a F1.4/2.5-

6mm lens. Cameras were connected to a video multiplexer (Panasonic Video Multiplexer, WJ FS 416) and a time-lapse videocassette recorder (Panasonic Time-Lapse VCR, AG-6540). Two cameras were spaced evenly and directly above the feed bunk in both the pre- and post-partum pens, and one camera was placed directly above the feed bunk of the maternity pen. Red Lights (100 W) were hung adjacent to the cameras to facilitate video recording at night. Individual cows were identified on the video recordings by unique alphanumeric symbols located on their back and sides. These symbols were placed on the cow using hair dye prior to entering the experiment. During both wk -1 and -2 relative to each cows actual calving date, 2 random days of video were evaluated providing 4 d of pre-partum video analysis for each cow. For each day of analysis the total number of times a cow was displaced from the feed bunk as well as the total number of times she displaced other individuals was recorded. A displacement from the feed bunk was noted when a cow's head came in contact with a cow who was feeding, such that the feeding cow withdrew its head from the feed bunk. The number of times a cow was displaced from the feed bunk was expressed as a percentage of the time spent feeding, in order to account for cows being displaced more often simply because they spent more time at the bunk.

### Individual Animal Factors

Body condition score (BCS; 1 to 5 following Wildman et al. 1982) was scored on d-20  $\pm$  2 and d-10  $\pm$  2 (relative to expected calving date) and every 3 d from calving until d+21. Body weights (BW) were measured at three times: d-20 ( $\pm$  2), d+2 ( $\pm$  2) and d+22 ( $\pm$  2). BW were determined by averaging 3 weight measurements taken over 3 consecutive days. Calving difficulty was recorded as either assisted (easy pull or difficult pull) or unassisted. Retained placenta was diagnosed if 24 h after calving the placenta

was observed hanging from the vaginal area. Cows with retained placenta were treated with penicillin for 3 consecutive days as per standard operating procedures on the farm. After calving daily rectal body temperatures (BT) were taken immediately after the morning milking using a digital thermometer (GLA M525/550, GLA Agricultural Electronics, San Luis Obispo, CA, USA). An examination of the udder and milk for clinical signs of mastitis (i.e. hard quarter, heat or swelling, clots in milk or clear/yellow milk) was performed every 3 d post-partum until d+21.

The time of calving for each individual cow was determined from video recordings and used to express feeding, drinking and standing data such that the day of calving (d 0) started at the time the calf was born. The day before and after the day of calving (d-1 and d+1) was also adjusted accordingly, but data from all other days were calculated starting at midnight.

Milk production data was collected during the morning and afternoon milking. These daily milk yields were then averaged by wk of lactation for each individual animal.

## Metritis Diagnosis

Vaginal discharge (VD) was evaluated for each cow every 3 d post partum until d+21. These examinations took place immediately following the morning milking (0730 h to 0900 h). Manual vaginal examination has been validated and does not cause uterine bacterial contamination, provoke an acute phase protein response, or delay uterine involution (Sheldon et al., 2002). Prior to vaginal palpation, the vulva was thoroughly cleaned with paper towel soaked in an iodine solution (Prepodyne Scrub, Prentone) diluted in warm water, to remove any feces that could introduce bacteria into the vaginal canal. Discharge was removed by inserting a gloved hand into the vaginal canal up to the cervix; any discharge present in this location was removed for visual

evaluation. The appearance and smell of the discharge was evaluated and assigned to a category based on the scoring system used by Urton et al. (2005) and adapted from Dohmen et al. (1995): no mucus or clear mucus = 0; cloudy mucus or mucus with flecks of pus = 1; mucopurulent ( $\leq 50\%$  pus present) and foul smelling = 2; purulent ( $\geq 50\%$  pus present) and foul smelling = 3; and putrid (red/brown color, watery, foul smelling) = 4.

### Determination of Health Status and Cow Participation in Study

Cows were classified as having severe metritis if identified as having at least one VD score of 4 and one recording of fever ( $\geq 39.5^{\circ}\text{C}$ ). Cows were classified as having mild metritis if they had a VD score of 2 or 3 with or without the presence of a fever. These definitions are similar to the clinical definitions of puerperal and clinical metritis defined by Sheldon et al. (2006). Healthy cows were defined based on the following criterion: maximum VD scores of 0 or 1, no post partum fever and no clinical signs of disease. There were 12 cows diagnosed with mild or severe metritis that also showed clinical symptoms of another infectious or metabolic disorder (i.e. mastitis as described above, or milk fever and ketosis as diagnosed by the herd veterinarian); these 12 animals were removed from all the study. Cows with retained placenta remained in the study as this disorder is strongly related to the incidence of metritis (Curtis et al., 1985; Kaneene and Miller, 1995; Bruun et al., 2002). Following removal of cows that failed to meet the above criteria 62 cows participated in the study: 12 severely metritic (5 primiparous and 7 multiparous), 27 mildly metritic (12 primiparous and 15 multiparous) and 23 healthy (5 primiparous and 18 multiparous).

## Statistical Analyses

All statistical analyses were performed with SAS (1999) using cows ( $n = 62$ ) as the experimental unit. Differences between actual and predicted calving dates resulted in differences in length of stay in the pre-partum pen ( $24 \pm 5$  d, mean  $\pm$  SD), but complete data was available from d-13 relative to the actual calving date. Feeding and drinking events were screened for normality and the presence of outliers by visual assessment of the distributions using Proc Univariate in SAS. Of 155779 feeding events 4.9% were identified as extreme outliers (more than 3 interquartile ranges from the box plot), likely due to the system not recording when the cow left the bin. Of 31775 drinking observations 1.6% were identified as outliers. Five experimental periods were defined for analysis: wk-2 (d-13 to d-8), wk-1 (d-7 to d-1), wk+1 (d+1 to d+7), wk+2 (d+8 to d+14) and wk+3 (d+15 to d+21). Data for the day of calving (d 0) was not included in an experimental period; however, this day was used for the descriptive analysis of behaviour around calving. Differences in feeding, drinking, DMI, and water intake between healthy cows versus severely metritic cows and healthy cows versus mildly metritic cows were analyzed by period using the contrast statement in a mixed model in SAS where health status (healthy, severely metritic or mildly metritic) and parity (primiparous or multiparous) were treated as fixed effects. DMI was also considered as a % of BW; however, these results did not differ from the results obtained using the unadjusted values, so only unadjusted DMI is reported below. Prior to this analysis, period means of heifers and cows in each health category were assessed separately to determine if any obvious health status  $\times$  parity interactions were present. This preliminary analysis did not reveal any obvious interactions; therefore, this effect was not included in the mixed models discussed above due to there being an insufficient

sample size of heifers and cows in each health category to perform a meaningful statistical analysis of the interaction between health status and parity.

The above analyses showed that the strongest differences among health groups in pre-partum feeding and drinking behaviour occurred during the wk prior to calving (wk-1), so the following analyses focused on this period. Differences among health categories in the distribution of feeding and drinking activity over a 24 hour period was determined using a mixed model in SAS using a heterogeneous autoregressive covariance structure (feed analysis) or a heterogeneous compound symmetry covariance structure (water analysis). A Type I analysis was used for fixed effects in the following order: parity, week, health status, and health status x week. Cow was treated as a random effect and week as a repeated measure.

Multivariate logistic regression was performed using the LOGISTIC procedure in SAS to model the effects of various intake and behavioural measures during wk-1 as well as BCS, BW, number of days on the close-up diet (i.e. time spent in the pre-partum pen), dystocia (assisted or unassisted calving) and parity on the presence or absence of severe metritis. Logistic models were used to fit one covariate at a time; variables with  $P < 0.2$  were then used to construct a complete logistic model. Variables included in this model were dystocia, days on close-up diet, and wk-1 DMI, feeding time and water intake. Feeding time and DMI were analyzed in separate logistic models due to the significant relationship between these two variables ( $R^2 = 0.60$ ,  $P < 0.001$ ). Variables were included into the model using a forward stepwise procedure based on a selection criterion of  $P < 0.05$ . Water intake during wk-1 did not meet the selection criteria for inclusion when included in either the DMI or feeding time model and was removed from the logistic analysis. A Type I analysis of the main effects based on estimated coefficients and their SE and the Likelihood Ratio and Wald tests resulted in the

following order of entry: dystocia, days on close-up diet, wk-1 DMI (or wk-1 feeding time).

Differences in social behaviour during the pre-partum period between healthy and mildly metritic cows and healthy and severely metritic cows were tested using t-tests. This test was based on a sub-sample of 36 cows; the 12 severely metritic cows and 12 cows from both the healthy and mildly metritic group matched to the severely metritic cows for parity and day of calving.

Differences in milk production during the 3 wks following calving, between healthy, mildly metritic and severely metritic cows were tested using a mixed model in SAS with a compound symmetry covariance structure and a Type I analysis of the following fixed effects in this order: parity, day, health status, and health status x day. Cow was treated as a random effect and day as a repeated measure.

## RESULTS

Of the 12 cows that were identified as having severe metritis, 7 also had retained placentas (58%), while only 2 of the 27 cows (7%) identified with mild metritis had retained placentas. The average number of days from calving to the first signs of pathological discharge ( $VD \geq 2$ ) was  $5.3 \pm 1.9$  d (mean  $\pm$  SD) for cows with severe metritis and  $9.1 \pm 3.9$  d for cows ( $P < 0.001$ ) with mild metritis. The average pre-partum BCS ( $\pm$  SD) of healthy, mildly metritic and severely metritic cows was  $3.38 \pm 0.18$ ,  $3.32 \pm 0.24$  and  $3.40 \pm 0.15$ , respectively. Mildly metritic and severely metritic cows produced on average 5.7 and 8.3 kg less milk per day ( $P < 0.001$ ) during the first 3 wks of lactation relative to healthy cows, respectively. The analysis of milk production also indicated an interaction between health status and day relative to calving ( $P = 0.02$ )

whereby the differences in milk production between the 3 health categories became greater as the days relative to calving increased (Figure 2.1).

## DMI and Feeding Behaviour

Severely metritic cows consumed less feed than healthy cows beginning 2 wks before calving and continued to consume less DM throughout the 4 remaining wks of the study (Table 2.1). Cows with mild post-partum metritis ate less DM relative to healthy animals during the wk prior to calving and the entire 3 wks post partum period (Table 2.1). Cow parity only affected DMI during wk-2 when primiparous cows consumed less feed than multiparous cows ( $P = 0.04$ ), and during wk+2 when primiparous cows consumed more feed ( $P = 0.05$ ); there was no effect of parity at any other experimental period.

Between d-7 to d-2, healthy, mildly metritic and severely metritic cows decreased their average daily DMI at a rate of 0.15, 0.21 and 0.33 kg/d, respectively (Figure 2.2 A). Not surprisingly, there was a dramatic drop in the DMI on d-1 (relative to the day before) for the healthy, mildly metritic and severely metritic cows (33%, 35% and 31%, respectively), followed by an increase in DMI (28%, 49% and 40%, respectively) in the 24h after calving. Of particular interest was that all 3 health groups again decreased their DMI by 11%, 13% and 34%, respectively on d +1.

Similar to the results observed for DMI, cows diagnosed with severe metritis spent less time feeding than healthy cows during each of the 5 wks, with differences most pronounced during the wks before and after calving (Table 2.1). Cows with mild metritis tended to have lower feeding times during the wk before calving relative to healthy cows, and had lower feeding time during wk+1. There was also a tendency for cows diagnosed with mild metritis to spend less time eating during wk+2. Irrespective of

health status primiparous cows had longer feeding times than multiparous cows ( $P \leq 0.01$ ). Similar to the results for DMI, cows progressively decreased their feeding times between d-7 to d-2 at a rate of 2.6, 4.0 and 4.8 min/d for healthy, mildly and severely metritic cows, respectively (Figure 2.2 B). On the day before calving feeding times dropped dramatically for healthy, mildly and severely metritic cows (33, 35 and 30 % decrease, respectively) relative to d-2. However, in contrast to the results obtained from DMI there was no increase in time spent feeding on the day of calving. Although healthy and mildly metritic cows showed relatively stable feeding times during the days around calving followed by a steady increase in daily feeding time after d+1, cows diagnosed with severe metritis further reduced their daily feeding times between d-1 and d+2 at a rate of 13.6 min/d (Figure 2.2 B). Feeding time was positively related to DMI, especially for cows with severe metritis (pre-partum period, i.e. d-13 to d-1,  $R^2 = 0.36, 0.41$  and  $0.64$  for healthy, mildly and severely metritic cows, respectively,  $P < 0.001$ ; post-partum period, i.e. d-1 to d+21,  $R^2 = 0.67, 0.69$  and  $0.81$  respectively,  $P < 0.001$ ).

There were no differences in feeding rate relative to healthy cows during any of the 5 experimental periods for either category of metritic cows (Table 2.1). While feeding rate remained relatively stable during the 2 wks before calving, there was a 46, 37 and 50% increase in feeding rate for healthy, mildly metritic, and severely metritic cows, respectively, on the day of calving (data not shown). Primiparous cows ate slower than multiparous cows ( $P \leq 0.02$ ) throughout the course of the study.

Analysis of the diurnal pattern of intake and feeding time during the wk before calving showed differences in hourly DMI and feeding time between healthy, and mildly or severely metritic dairy cows ( $P \leq 0.003$ ; Figure 2.3 A). We observed a health status by hour interaction for DMI ( $P = 0.03$ ) indicating that differences in hourly intake were affected by time of day. The strongest differences in DMI between health categories

occurred in the hours immediately following morning and afternoon fresh feed delivery. We noted a similar health status by hour interaction ( $P = 0.06$ ) for feeding time. There were no differences in hourly feeding rate between the 3 health categories during the wk before calving ( $P = 0.73$ ).

## Drinking Behaviour and Water Intake

Cows identified with severe or mild post-partum metritis consumed less water than healthy cows in the 3 wks post partum and showed a tendency to consume less water during the wk before calving (Table 2.2). For healthy, mildly and severely metritic cows, respectively, there was a 20, 19 and 7 % decline in water intake on d-1, followed by a 67, 89 and 44% respective increase in water intake on the day of calving (Figure 2.4). Water intake dropped by 15% for healthy and mildly metritic cows and by 13% for severely metritic on d+1. Unlike the pattern observed for DMI there was no obvious decline in water intake in the wk before calving (Figure 2.4). Primiparous cows consumed less water than multiparous cows ( $P \leq 0.04$ ) throughout the study.

There were no differences between healthy cows and those diagnosed with either severe or mild post-partum metritis in total time spent drinking per day (Table 2.2), and no effect of parity on time spent drinking. There were weakly positive relationships between drinking time and total amount of water consumed for healthy, mildly metritic and severely metritic cows during the pre-partum (d-13 to d-1;  $R^2 = 0.23$ , 0.03 and 0.07, respectively,  $P < 0.001$ ) and post-partum periods (d-1 to d+21;  $R^2 = 0.10$ , 0.08 and 0.26, respectively,  $P < 0.001$ ).

There were no differences in the drinking rate between healthy cows and those later diagnosed with severe metritis. However, mildly metritic cows had slower rates of

water consumption than healthy cows (Table 2.2). Primiparous cows drank more slowly than multiparous cows ( $P \leq 0.05$ ).

The diurnal pattern of water intake (Figure 2.3 B) and drinking rate (data not show) during the wk before calving was different among the 3 health categories ( $P \leq 0.007$ ). Analysis of drinking rate indicated an interaction between health status and hour ( $P < 0.001$ ). Differences in drinking rate were most pronounced following the delivery of fresh feed with healthy cows drinking faster during these periods. There were no differences in hourly drinking time among the 3 health categories during the wk before calving ( $P = 0.37$ ).

## Logistic Regression

Logistic regression showed that dystocia, days on close-up diet, wk-1 DMI and wk-1 feeding time were associated with an increased risk of developing severe metritis. The odds of cows being diagnosed with severe metritis increased by 15.8 when calving was assisted relative to when it was not [ $P_{\text{Wald}} = 0.004$ ; 95% confidence interval ( $CI_{95}$ ) = 2.36, 105.49] and increased by 1.19 for every day less that a cow consumed the close-up diet prior to calving ( $P_{\text{Wald}} = 0.04$ ;  $CI_{95} = 1.01, 1.41$ ). The odds of severe metritis increased by 2.87 for every 1 kg decrease in DMI during the wk before calving ( $P_{\text{Wald}} = 0.02$ ;  $CI_{95} = 1.16, 7.09$ ). This logistic model (including dystocia, days of close-up diet and DMI during wk-1) accounted for 58% of the variation in risk of severe metritis ( $R^2 = 0.58$ ,  $P_{\text{Likelihood Ratio (LR)}} < 0.001$ ). The odds of severe metritis increased by 1.72 for every 10 min decrease in feeding time during wk-1 ( $P_{\text{Wald}} = 0.02$ ;  $CI_{95} = 1.08, 2.75$ ). In this logistic model that substituted feeding time for DMI, 54% of the variation in risk of severe metritis was accounted for ( $R^2 = 0.54$ ,  $P_{\text{LR}} < 0.001$ ).

## Social behaviour

During the 2 wks before calving healthy cows displaced others from the feed bins on average  $16.8 \pm 1.74$  times/d compared to severely metritic cows who only displaced others on average  $12.2 \pm 1.58$  times/d ( $P = 0.06$ ). There was no difference between healthy and mildly metritic cows in terms of how often they displaced others at the feed bunk during the pre-partum period. After adjusting for the time each cow spent feeding per day there were no differences between the health categories in terms of the number of times individuals were displaced from the feed bunk. Multiparous cows were more aggressive at the feed bunk displacing others on average  $17.9 \pm 1.20$  times/d relative to primiparous cows that displaced others on average  $11.5 \pm 1.52$  times/d ( $P = 0.002$ ). Primiparous cows were displaced from the feeding area more often than they displaced others while multiparous cows displaced others more often than they were displaced by other cows ( $P < 0.001$ ).

## DISCUSSION

In this study 50 % of dairy cows (51 out of 101) were diagnosed as either mildly or severely metritic based on our definitions for this disorder; however, 24 % of these animals also showed clinical symptoms of an additional post-partum disorder. Although numerous reports exist with reference to uterine infection, there is a wide range of criteria used to diagnose and classify this disease, and in some cases diagnostic criteria are poorly described making comparisons of incidence rates difficult. This variation in diagnostic methods among studies no doubt contributes to the wide range in the incidence rates of metritis/endometritis reported in the literature (14.8%, Borsberry and

Dobson, 1989; 7.6%, Gröhn et al., 1995; 53%, Gilbert et al., 2005; 16.9%, LeBlanc et al., 2002; 69%, Urton et al., 2005).

Milk production was lower in those cows identified with severe and mild metritis in our study during the first 3 wks after calving. The reduction in milk production is likely a consequence of the decreased DMI and water intake observed in these animals relative to those that remained healthy after calving. Other studies have also reported that cows diagnosed with metritis within the first few wks after calving showed lower milk yields particularly during early lactation (Deluyker et al., 1991; Rajala and Gröhn, 1998).

A variety of risk factors have been associated with metritis with the most frequently cited factors being retained placenta and calving difficulty (Curtis et al., 1985; Kaneene and Miller, 1995; Bruun et al., 2002). Similar to these reports we also noted that 58% of severely metritic cows also had retained placentas, while only 7% of the mildly metritic cows and none of the healthy cows were diagnosed with a retained placenta. In addition we showed that cows were 15.8 times more likely to develop severe metritis if they had an assisted calving. Calving difficulty increases trauma to the uterine wall and increases the susceptibility to disease by increasing the risk of harmful bacteria entering the reproductive tract (Bruun et al., 2002).

Days spent consuming a close-up diet was related to post-partum health status of the animals observed in this study. Although negative effects on performance and health have been associated with feeding a close-up ration for longer than the recommended 3-wk period (Mashek and Beede, 2001), to our knowledge this is the first study to show that fewer days on the close-up TMR increased risk of metritis. The NRC (2001) recommend feeding a diet containing approximately 1.25 Mcal/kg of  $NE_L$  (far-off diet) until 21 d pre-partum and then 1.54 to 1.62 Mcal/kg of  $NE_L$  (close-up diet) for the

last 3 wks preceding parturition. The close-up ration is specifically formulated to provide the necessary protein, energy and minerals levels required to make the metabolic and physiological adaptations necessary for parturition and the onset of lactation (NRC, 2001). The cows that went on to develop severe metritis in the present study were already consuming less DM than cows that remained healthy, and may have been further compromised nutritionally by spending fewer days on the diet. These results would support the recommendation to ensure adequate pre-partum nutrition in order to maintain post-partum health.

In the present study pre-partum BW and BCS, as well as parity, were not useful as predictive measures for severe post-partum metritis. Numerous studies have attempted to relate these factors to post-partum health but results have been inconsistent. For example, Gröhn et al. (1990) reported no associations between metritis risk and parity while Markusfeld (1984) reported first time calvers as being at the greatest risk for metritis. Bruun et al. (2002) suggested that both 1<sup>st</sup> parity and cows of 3<sup>rd</sup> parity or higher were at the greatest risk for this disease. In each of these studies cows were maintained under varying management conditions and this may explain the lack of consistency in how parity relates to post-partum health status. For example group size, composition and frequency of mixing new herd mates may influence heifers and cows differently and consequently their susceptibility to disease. Contreras et al. (2004) examined BCS during transition and subsequent effects on health, and reported increased rates of retained placenta, ketosis and milk fever in cows that had a BCS  $\geq$  3.25 at dry off. Kaneene and Miller (1995) reported that over-conditioned and under-conditioned cows were at greater risk for metritis. Interestingly, DMI has been shown to decrease linearly with increasing BCS during the pre-partum period (Hayirli et al., 2002), which may partially explain the relationship between post-partum health and pre-

partum BCS. However, in the present study we found no relationship between pre-partum BCS and pre-partum intake. This may be attributed, in part, to a limited sample size, and may also be a consequence of low variation in BCS of the cows used in the current study.

To our knowledge this is the first study to examine the relationship between water intake and post-partum health status in transition dairy cows. When investigated as a single factor, water intake did have some predictive ability in identifying animals at risk for metritis, but when included in a logistic model with either DMI or feeding time this variable was not significant. This was not surprising given that both feeding measures were much stronger pre-partum predictors of severe metritis than water intake. Over the course of the study differences in water intake between mildly metritic and healthy cows were more significant than differences in water intake between severely metritic and healthy cows. A larger sample size of severely metritic animals is likely needed in order to detect significant differences in pre-partum water intake between health categories, as pre-partum differences in water intake among the 3 health categories were more subtle than the differences observed for pre-partum DMI and feeding time.

An interesting result of this study was that during the wk before calving water intake differed little between severely metritic and healthy animals, despite the fact that DMI is generally correlated with free water intake (Murphy, 1992). When looking at the diurnal pattern of water intake during the wk before calving, we observed that cows that went on to develop severe metritis consumed less water during peak feeding times (period following fresh feed delivery) however these animals appeared to compensate by consuming more water than healthy cows at other hours of the day. An explanation for why these animals may be able to maintain higher levels of water intake during the

pre-partum period may be due to the fact that the time needed to consume their water requirements is considerably lower than the time needed to consume their DMI requirements. Consequently aggressive interactions at the water trough may have less impact on water intake than they do on feed intake.

Although this study showed positive relationships between drinking time and water intake during both pre-partum and post-partum periods, only 26% of the variation in water intake could be explained by drinking time, suggesting that cows vary greatly in the time spent at the water trough. A limitation of the Insentec system for monitoring drinking and feeding time is that the system records the time that a cow spends at the feed/water bin and not the amount of time that the cow is actually consuming water or feed. Because cows require only minutes to consume their water requirements (Huzzey et al., 2006), it is possible that this variation in drinking time is actually due to variation in the time cows spend at the water trough but not drinking.

In this study pre-partum feeding time and DMI were best able to identify cows at risk for severe metritis. These results complement other studies, which have examined the relationship between feeding behaviour and health status. An earlier study by our research group showed that the odds of a positive diagnosis of acute metritis increased by 1.57 for every 10 min decrease in feeding time during the two wks before calving (Urton et al., 2005). This value compares well with the results of the current study, showing a 1.72 increase in risk for every 10 min decrease in feeding time during the wk before calving. Work completed by Quimby et al. (2001) with feedlot steers indicated that reduced feeding behaviour can be used to detect animal morbidity approximately 4.1 d earlier than identification by pen riders. Hammon et al. (2006) reported lower DMI, relative to healthy animals, during the 2 wk period before calving for cows that went on to develop puerperal metritis or sub-clinical endometritis.

The results of the current study and those of previous studies provide clear evidence that reduced feeding time and DMI increases the risk of cows developing infectious disease after calving. However, whether a reduction in intake and feeding time before calving is a cause of post-partum infectious disease or an effect of a pre-existing condition, is not known. Ketotic environments (i.e. low concentrations of plasma glucose, and high concentrations of NEFA and BHBA) have been shown to impair immune function through a variety of pathways (Sato et al., 1995; Suriyasathaporn et al., 1999; Zdzisinska et al., 2000). Hammon et al. (2006) reported that cows that went on to develop puerperal metritis and sub-clinical endometritis, had a greater degree of immunosuppression relative to healthy animals as measured by neutrophil function. Compromised immune status as a result of inadequate nutrition during the pre-partum period may increase a cow's susceptibility to post-partum metritis; however, the underlying reason for the reduction in feeding behaviour in some cows and not others is not known.

The reduction in feeding time and intake during the pre-partum period may be a consequence of a pre-existing condition within the cow. Our investigations into the behaviour of dairy cows during the pre-partum period provide the first evidence that social behaviour may play an important role in disease susceptibility in dairy cattle. During the wk before calving cows later diagnosed with severe metritis engaged in fewer aggressive interactions at the feed bunk (i.e. displaced others from the feed bunk less often) and had reduced feeding and drinking times especially during the periods following fresh feed delivery. Previous work has shown that the motivation to feed is greatest immediately following the delivery of fresh feed, and competition at the feed bunk is greatest at this time (DeVries and von Keyserlingk, 2005). Cows that were diagnosed with severe metritis after calving appear to be less motivated to compete for

access to the feed prior to calving. A low propensity to compete for access to feed may indicate that these cows are socially subordinate and unwilling to engage in interactions with more dominant individuals.

It is generally accepted that DMI declines over the wk before calving, contributing to the negative energy balance after parturition (Grummer et al., 1995; Dann et al., 1999; Grummer et al., 2004). However, these reports often fail to consider post-partum health status. The aim of most transition cow management programs is to improve DMI during the pre-partum period, thereby, minimizing the degree of negative energy balance post-partum. The results of the current study show only some cows exhibit a dramatic decline in DMI the wk before calving. Cows that remained healthy throughout the entire transition period showed little decline in DMI in the period before calving. In contrast, cows with severe post-partum metritis showed a notable decline in daily DMI beginning 1 wk prior to calving.

The reduction in pre-partum feeding activity experienced by some individuals may also be a consequence of differences in coping strategies between individuals. There are numerous changes that occur during the transition period including regrouping of animals. The group structure within the pre-partum pen in this study was dynamic with cows entering and leaving the group on average every 3 d. Unfortunately this dynamic group structure makes classification of animals in terms of social hierarchy difficult, as this is dependant on an individual's relationship to others housed in the same pen (Langbein and Puppe, 2004). Socially subordinate animals may not be able to cope with frequent restructuring of the social hierarchy resulting in reduced feeding time, DMI and avoidance behaviour in response to social confrontations, putting these cows at greater risk of nutritional deficiencies that impair immune function and increase susceptibility to disease. In the present study, groups consisted of both heifers and

cows, and these animals shared access to the same feeders. Both of these factors can influence social dynamics within the group (Phillips and Rind, 2001; Huzzey et al., 2006) with the socially subordinate cows being most affected.

These findings provide further evidence that differences in feeding and social behaviour exist between cows and that these differences influence the risk of cows succumbing to disease after calving. Our findings indicate that new work is now required to better understand transition cow responses to management practices such as regrouping, and how this affects their susceptibility to post-partum disease.

## CONCLUSIONS

The results of this study indicate that both DMI and feeding behaviour, particularly during the wk before calving, can identify cows at risk for post-partum metritis. We also provide the first evidence that cows that go on to develop post-partum metritis, engage in fewer aggressive interactions at the feed bunk during the wk prior to calving and avoid the feed bunk during periods when competition for feed is highest. These results suggest that environmental and social factors may be important in determining the level of feeding activity in individual cows during the pre-partum period and subsequently may influence her post-partum health status.

## ACKNOWLEDGEMENTS

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	Period				
	wk -2	wk -1	wk +1	wk +2	wk +3
DMI (kg /d)					
Healthy	14.86 ± 0.45	14.32 ± 0.52	14.81 ± 0.67	16.45 ± 0.66	17.41 ± 0.72
Mild Metritis	14.28 ± 0.39	12.72 ± 0.45*	12.19 ± 0.58**	13.92 ± 0.57**	15.45 ± 0.62*
Severe Metritis	13.09 ± 0.59*	11.22 ± 0.68***	8.75 ± 0.87***	12.08 ± 0.86***	13.61 ± 0.93**
Feeding Time (min/d)					
Healthy	214.06 ± 8.35	192.29 ± 8.91	155.46 ± 7.42	179.22 ± 9.20	188.84 ± 9.90
Mild Metritis	203.76 ± 7.24	170.66 ± 7.72 <sup>†</sup>	130.76 ± 6.43*	158.41 ± 7.98 <sup>†</sup>	176.20 ± 8.58
Severe Metritis	184.75 ± 10.89*	145.97 ± 11.62**	91.55 ± 9.67***	147.10 ± 12.00*	160.49 ± 12.91 <sup>†</sup>
Feeding Rate (kg DMI/min)					
Healthy	0.074 ± 0.003	0.078 ± 0.003	0.100 ± 0.004	0.098 ± 0.003	0.097 ± 0.003
Mild Metritis	0.077 ± 0.002	0.082 ± 0.003	0.102 ± 0.003	0.097 ± 0.003	0.096 ± 0.003
Severe Metritis	0.080 ± 0.004	0.086 ± 0.004	0.105 ± 0.005	0.089 ± 0.005	0.092 ± 0.004

Significance level for difference between mildly metritic and healthy cows and severely metritic and healthy cows

<sup>†</sup> P < 0.10

\* P < 0.05

\*\* P < 0.01

\*\*\* P < 0.001

**Table 2.1.** Least squared means ± SE for DMI, feeding time and feeding rate for healthy Holstein dairy cows and those with severe and mild post-partum metritis during 5 experimental periods (wk -2, -1, +1, +2 and +3).

	Period				
	wk -2	wk -1	wk +1	wk +2	wk +3
Water Intake (kg/d)					
Healthy	39.65 ± 1.64	37.82 ± 1.64	63.66 ± 2.94	77.92 ± 2.59	82.36 ± 2.41
Mild Metritis	34.50 ± 1.42*	32.78 ± 1.42*	54.66 ± 2.54*	62.42 ± 2.24***	71.05 ± 2.09***
Severe Metritis	36.51 ± 2.13	32.94 ± 2.14 <sup>†</sup>	52.29 ± 3.83*	68.53 ± 3.37*	68.95 ± 3.14**
Drinking Time (min/d)					
Healthy	9.36 ± 1.01	8.65 ± 0.85	11.51 ± 1.05	13.37 ± 1.39	13.02 ± 1.15
Mild Met	10.15 ± 0.88	9.47 ± 0.74	13.43 ± 0.91	15.45 ± 1.20	15.10 ± 1.00
Severe Met	10.68 ± 1.32	8.94 ± 1.11	9.55 ± 1.37	15.45 ± 1.81	12.44 ± 1.50
Drinking Rate (kg/min)					
Healthy	5.88 ± 0.64	5.81 ± 0.60	6.46 ± 0.48	6.87 ± 0.56	7.41 ± 0.60
Mild Metritis	4.26 ± 0.55 <sup>†</sup>	4.25 ± 0.52*	4.58 ± 0.42**	4.70 ± 0.48**	5.33 ± 0.52**
Severe Metritis	4.78 ± 0.83	4.89 ± 0.78	6.50 ± 0.63	5.51 ± 0.73	6.62 ± 0.78

Significance level of difference between mildly metritic and healthy cows and severely metritic and healthy cows

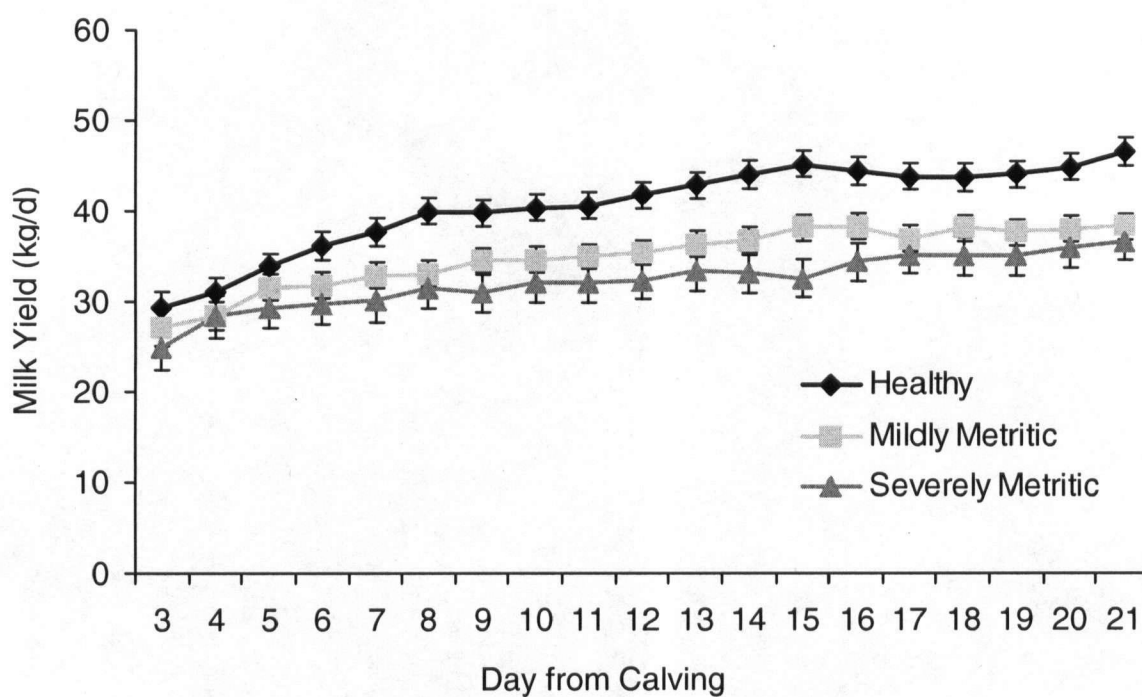
<sup>†</sup> P < 0.10

\* P < 0.05

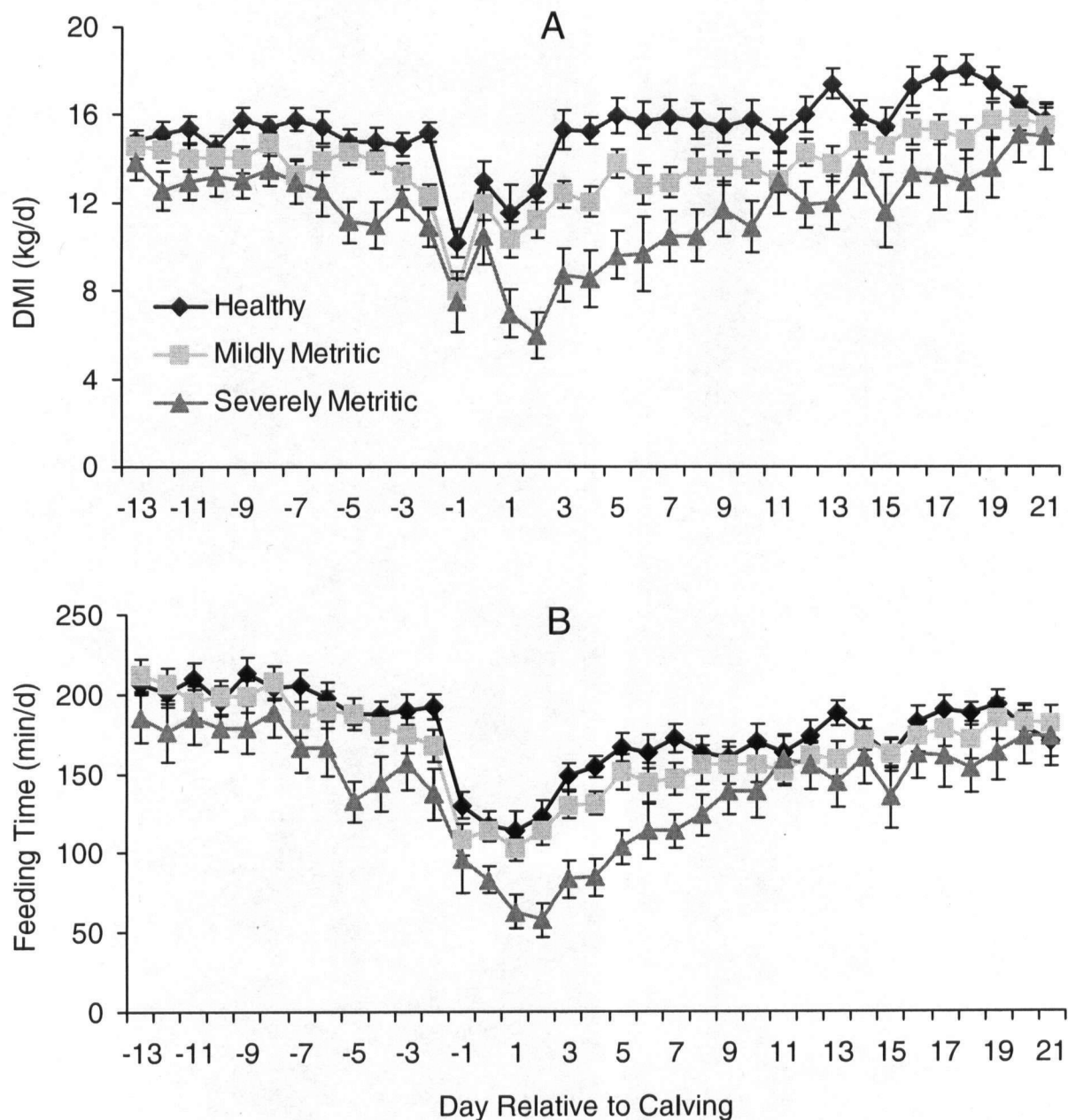
\*\* P < 0.01

\*\*\* P < 0.001

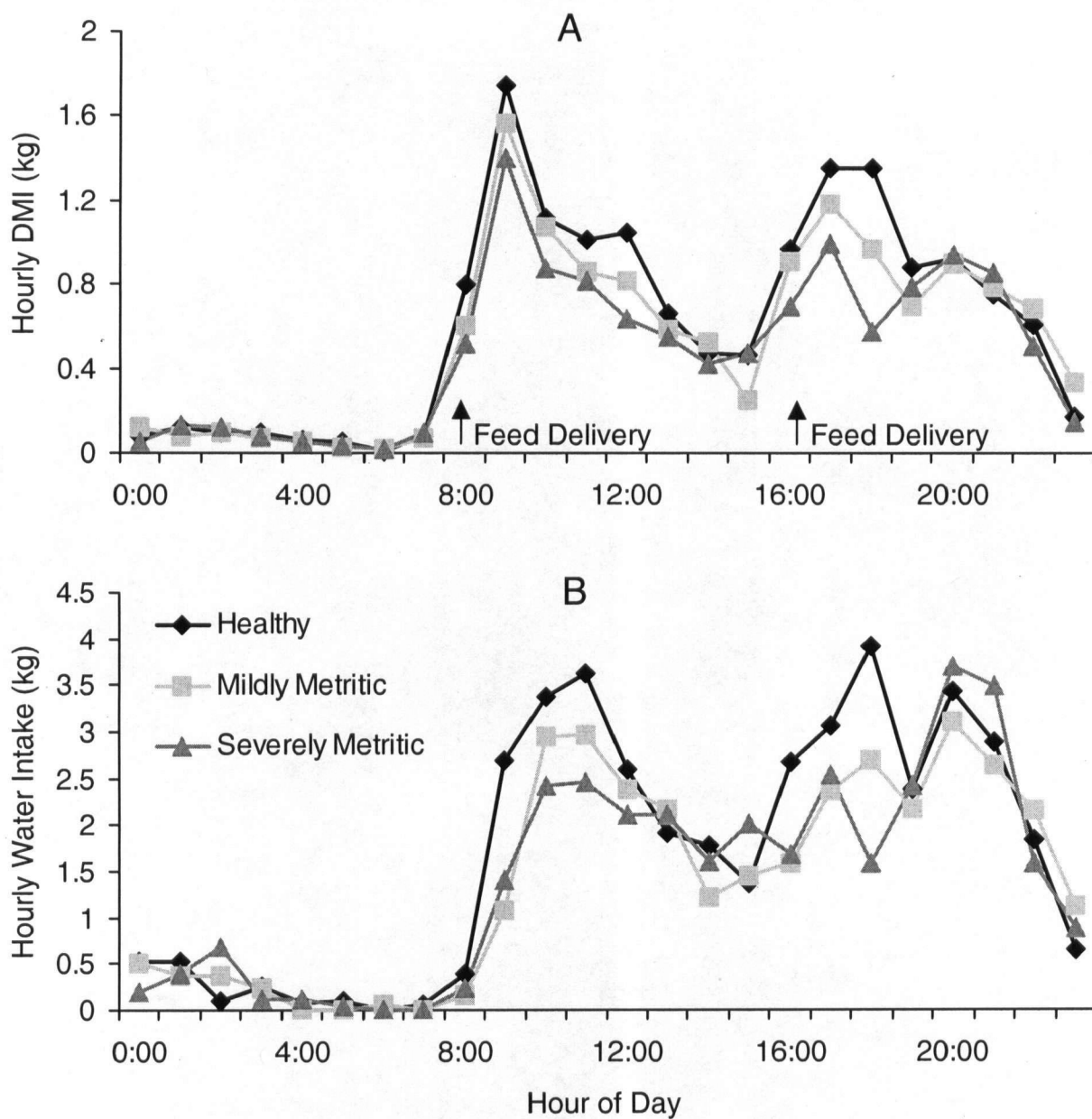
**Table 2.2.** Least squared means ± SE for water intake, drinking time and drinking rate for healthy Holstein dairy cows and those with severe and mild post-partum metritis during 5 experimental periods (wk -2, -1, +1, +2 and +3).



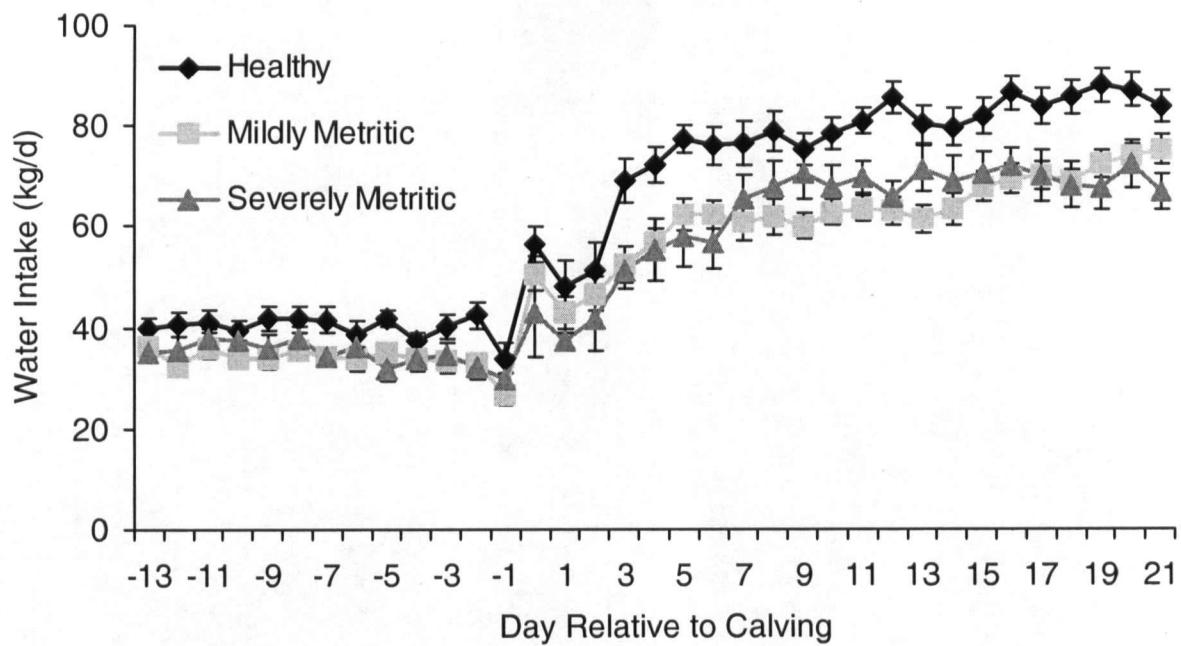
**Figure 2.1.** Least squared mean ( $\pm$  SE) milk yield (kg/d) during the first 3 wks of lactation for healthy (n=23), mildly metritic (n=27) and severely metritic (n=12) Holstein dairy cows.



**Figure 2.2.** Average ( $\pm$  SE) daily DMI (kg/d; A) and feeding time (min/d; B) of healthy ( $n=23$ ), mildly metritic ( $n=27$ ) and severely metritic ( $n=12$ ) Holstein dairy cows from 13 d before until 21 d after calving.



**Figure 2.3.** Diurnal pattern of hourly feed consumption (Least Squared (LS) Mean DMI  $\pm$  SE; A) and water intake (LS Mean water kg  $\pm$  SE; B) for healthy (n=23), mildly metritic (n=27) and severely metritic (n=12) Holstein dairy cows during the wk before calving (d-7 to d-2).



**Figure 2.4.** Average ( $\pm$  SE) daily water intake (kg/d) of healthy (n=23), mildly metritic (n=27) and severely metritic (n=12) Holstein dairy cows from 13 d before until 21 d after calving.

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### CHAPTER 3: GENERAL CONCLUSIONS

The research described in this thesis provides the first experimental evidence that social behaviour before calving may help to identify cows at risk for disease after calving. Cows that developed severe metritis after calving engaged in fewer aggressive interactions at the feed bunk and avoided the feeding areas during peak feeding times, when bunk occupancy was the highest. When faced with stressful situations individuals can use different strategies to cope (Mendl et al., 1992; Wechsler, 1995; Koolhaas et al., 1999). In a review by Koolhaas et al. (1999), two different coping strategies were discussed: proactive coping, involving an active response to a social challenge including high activity and aggression; and reactive coping, characterized by immobility and low aggression. In this study cows that engaged in fewer aggressive interactions and avoided the feed bunk during peak occupancy periods appeared to demonstrate behaviours consistent with a reactive coping strategy. Sustained over-activation of neuroendocrine systems related to reactive coping styles (i.e. greater activation of the hypothalamic-pituitary-adrenal (HPA) axis with higher levels of circulating cortisol) have been shown to result in immunosuppression in dairy cows (Hopster et al., 1998) and pigs (Tuchscherer et al., 2004). Elevated and sustained HPA axis activity may have occurred before calving in the cows that developed severe metritis, unfortunately this cannot be confirmed as we did not monitor HPA activity. Hammon et al. (2006), however, showed that immune status is compromised before calving in cows that develop metritis after calving.

Future research in this area should examine whether individual dairy cows display consistent behavioural strategies to aversive environments such as competitive feeding environments, and determine how these strategies relate to the health status of

the cow during the transition period. Some individuals may be especially sensitive to environmental stressors. In the study by Hopster et al. (1998), cows were identified as being high responders to stress (high cortisol concentrations) or low responders to stress (low cortisol concentrations), in response to a novel environment test. One year later these same animals were subjected to social isolation and were given an injection of endotoxin to assess various aspects of their immunocompetence. Cows that had previously been identified as high responders, showed the strongest stress responses to isolation and handling (higher body temperature, greater increases in cortisol, and more vocalizations) and were less successful in mounting an immune response to the endotoxin compared to the cows that had previously been identified as low responders. If we can identify individual cows that are highly susceptible to stress, producers may be able to alter management practices to minimize stress for these animals during the transition period, for example, by providing ample space at the feed bunk to minimize competition for feed.

The results described in this thesis demonstrate that changes in feeding time and intake occurred nearly 3 wks before clinical signs of severe metritis. These findings are similar to findings showing that feeding time (Urton et al., 2005) and DMI (Hammon et al., 2006) before calving are lower in cows that develop metritis relative to cows that remain healthy. Although the reduction in feeding activity likely contributed to the increased risk of metritis, the reason for this suppression in intake and behaviour is still unknown.

Management of intensively housed dairy cattle has been shown to influence the behavioural patterns of dairy cows [i.e. mixing or re-grouping (Tennessen et al., 1985), housing heifers and cows together (Bach et al., 2006), and overstocking (Huzzey et al., 2006)]. Unfortunately, despite cows at transition being subjected to many of these

management procedures, research on these topics has focused on animals in peak to mid lactation. The impact of these management practices on the behaviour and health of cows around the time of calving is not known. In fact, there has been no other published work other than the study by Urton et al. (2005) that has considered the relationship between behaviour and disease during the transition period. Future work must now focus on understanding how behaviour, stress, and management practices interrelate, and how these in turn relate to disease after calving.

These results are relevant to dairy producers who need to reduce the incidence of disease around calving. If cows at risk for disease can be identified prior to the onset of illness there may be opportunities for management changes that either prevent the illness or reduce its severity. DMI is a useful predictor of metritis, but monitoring individual feed intake in commercial, group housed dairy cows is not practical due to the high cost of purchasing the necessary equipment. The work described in this thesis shows that changes in feeding behaviour (i.e. time spent at the feed bunk, pattern of bunk attendance) are also good predictors of metritis, and these would be easier to obtain on commercial farms.

For food security reasons, all cattle owners in Canada will be required to tag their calves with a unique radio frequency identification (RFID) transponder by 2008 (NLID, 2006), meaning that all producers will already possess half the technology required to monitor feeding activity. A simple, inexpensive antenna system at the feed bunk that could detect the presence of the RFID tags (i.e. cow presence at the feed bunk) would not be difficult to develop. This technology could be combined with a computer software program that would summarize individual cow bunk attendance on a daily basis, and advise producers which cows had the lowest bunk attendance times or had large fluctuations feeding activity over the course of a few days.

Once producers have identified the cows at risk for disease they need to know what to do with these animals. My current recommendation would be to err on the side of caution and manage these cows in ways that minimize or eliminate known environmental stressors. For example, high-risk cows should not be housed in over stocked pens that are known to have negative impacts on both the feeding and social behaviour of dairy cows (Huzzey et al., 2006). Further, management strategies that increase aggression among animals such as regrouping should be avoided (Hasegawa et al., 1997).

One factor that requires further discussion was the feeding system described in Chapter 2. The Insentec system captures detailed information on individual feeding behaviour and intake of group housed dairy cows, but creates a very different feeding environment than that provided by the feed barriers commonly used on commercial dairy farms (i.e. the headlock and post-and-rail feed barriers; see Illustration 3.1). The type of feed barrier used can affect both the social and feeding behaviour of dairy cows (Huzzey et al., 2006); consequently, the use of the Insentec system likely influenced these measures. A qualification of my research is that the values reported in Chapter 2 are specific for cows fed using the Insentec system. To illustrate this point, consider the average daily feeding time of healthy and severely metritic cows during the 2-wk period before calving (approximately 200 and 165 min/d, respectively) in my study relative to the feeding times reported by Urton et al. (2005) for a similar period before calving (85 and 62 min/d, respectively). In the study by Urton et al. (2005), cows used a post-and-rail feed barrier, and the electronic monitoring system required that cows be within 50 cm of the antenna incorporated into a mat under the feed. In that study, feeding time was only recorded when the head of the cow was in close proximity to the feed. In contrast, feeding time in my study was calculated as the total time elapsed between the

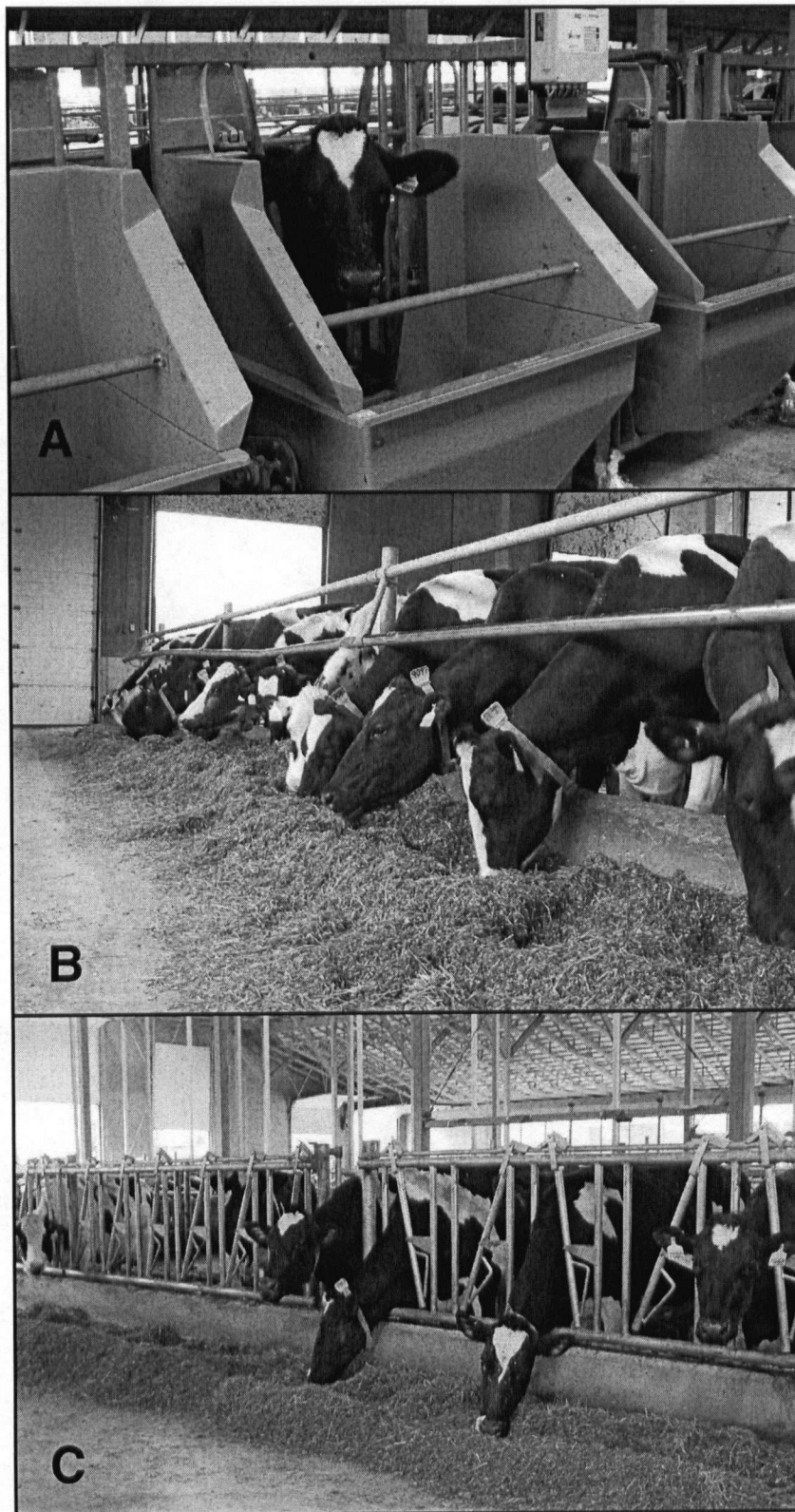
gate opening (allowing the cow access to the feed) and the gate closing. Although neither method of measuring feeding time directly represents the time cows spend consuming feed on commercial dairy farm, the results described in this thesis and in the study by Urton et al. (2005) provide clear evidence that cows that develop metritis after calving spend less time at the feed bunk before calving, relative to cows that remain healthy.

In this study the presence of sub-clinical disorders such as ketosis, hypocalcaemia and mastitis were not accounted for when classifying the cows into the 3 health categories (healthy, mildly metritic and severely metritic). Many health disorders associated with the transition period are related in their occurrence; for example, Whiteford and Sheldon (2005) showed that cows with a history of hypocalcaemia were more likely to have endometritis. Future work should therefore examine the relationship between behaviour during the transition period and the occurrence of other common post-partum diseases, including clinical and sub-clinical mastitis, ketosis and milk fever.

Finally, the relatively few animals in the severely metritic group compared to the other two health categories may have limited my ability to detect relationships. Although a difference in pre-partum water intake was detected between healthy and mildly metritic animals during the 2-wk period before calving, severely metritic cows only showed a tendency to consume less water during the wk before calving relative to healthy cows. A larger sample size would have provided more statistical power to detect differences in water intake between healthy and severely metritic cows during the period before calving.

Previous work on transition cow health has focused on nutritional strategies (i.e. altering the diet) as a means of preventing disease around calving yet the incidence rates of transition related diseases has continued to remain high. The results presented

in this thesis provide evidence that factors other than pre-partum nutrition, including feeding and social behaviour, are related to disease after calving. Management practices that limit the ability of some cows to access the diet offered may be just as important as how that diet is formulated. Future progress in this area must combine our understanding of nutrition, metabolism, physiology, immunology and behaviour to determine management strategies that will reduce the incidence of disease after calving.



**Illustration 3.1.** Pictures of the Insentec (A), post-and-rail (B) and headlock (C) feed barrier systems.

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