

FEEDING BEHAVIOUR IDENTIFIES DAIRY COWS AT RISK OF SUBCLINICAL  
KETOSIS DURING THE TRANSITION PERIOD

by

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## ABSTRACT

Cows diagnosed with subclinical ketosis (SCK) after calving are at increased risk of developing other diseases and compromised reproductive performance. The objective of this study was to determine if changes in voluntary feeding and social behaviours during the transition period, consisting of the 3 wk before and 3 wk after calving, could identify dairy cows at elevated risk of SCK during the week after calving. The feeding behaviours of 101 Holstein dairy cows were monitored from 3 wk before to 3 wk after calving. Ten animals were identified as having SCK by serum BHBA levels  $\geq 1000 \mu\text{mol/L}$  during wk +1, but were otherwise healthy. These animals were compared with 10 healthy animals, balanced for parity. Displacements at the feed bunk were measured during peak feeding times on 3 d during the week before calving. During the wk before calving and the 2 wk after calving, animals with SCK had lower dry matter intake (DMI), fewer visits to the feeder and spent less time at the feeder than healthy animals. For every 10 min decrease in average daily time spent at the feeder during the week before calving, the risk of SCK increased by 1.9 times. During the same week, a 1 kg decrease in average daily DMI increased the risk of SCK by 2.2 times. The largest increase in risk of SCK was associated with a 1 kg decrease in the change in average daily DMI from wk -2 to wk -1. During the week before calving, SCK animals initiated fewer displacements at the feed bunk when compared to animals that remained healthy after calving. The results of this study provide evidence that the amount of time spent feeding, DMI and social behaviour are associated with the development of SCK after calving. These results indicate that special consideration should be given to management and social factors that can negatively affect feeding behaviour during the transition period.

## TABLE OF CONTENTS

<b>ABSTRACT</b> .....	<b>ii</b>
<b>TABLE OF CONTENTS</b> .....	<b>iii</b>
<b>LIST OF TABLES</b> .....	<b>iv</b>
<b>LIST OF FIGURES</b> .....	<b>v</b>
<b>LIST OF ABBREVIATIONS</b> .....	<b>vi</b>
<b>ACKNOWLEDGEMENTS</b> .....	<b>vii</b>
<b>CO-AUTHORSHIP STATEMENT</b> .....	<b>viii</b>
<b>CHAPTER 1: INTRODUCTION</b> .....	<b>1</b>
THE TRANSITION PERIOD: CHANGES AND CHALLENGES .....	3
Changes at Transition: Physiology .....	3
Challenges at Transition: Management.....	5
COPING WITH CHANGE: ENERGY MANAGEMENT DURING THE TRANSITION PERIOD .....	6
SOURCES OF VARIATION IN FEED INTAKE .....	8
Animal Factors Influencing Variability .....	8
Management Factors Influencing Variability.....	11
KETOSIS: WHEN THERE JUST ISN'T ENOUGH ENERGY!.....	13
OBJECTIVE.....	15
HYPOTHESIS .....	16
REFERENCES .....	17
<b>CHAPTER 2: PREPARTUM FEEDING BEHAVIOUR IS AN EARLY INDICATOR OF SUBCLINICAL KETOSIS</b> .....	<b>24</b>
INTRODUCTION .....	24
METHODS .....	26
Animals, Housing and Diet.....	26
Behavioural Data Collection.....	26
Case Definition and Cow Participation in Study .....	27
Social Behaviour Data Collection .....	28
Statistical Analysis .....	29
RESULTS.....	31
DMI and Feeding Behaviour.....	31
Logistic Regression.....	32
Milk Production and Composition .....	32
Social Behaviour at the Feed Bunk .....	33
DISCUSSION .....	33
CONCLUSIONS.....	36
ACKNOWLEDGEMENTS .....	37
REFERENCES .....	41
<b>CHAPTER 3: GENERAL CONCLUSION</b> .....	<b>44</b>
FUTURE RESEARCH.....	46
REFERENCES .....	47

## List of Tables

<b>Table 1.</b> Least squares means ( $\pm$ SE) for milk composition of healthy cows (n=10) and those with SCK (n=10) during the 3 weeks following calving.....	38
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## LIST OF FIGURES

**Figure 1.** Least squares mean ( $\pm$ SE) for A) DMI, B) number of visits to the feeder and C) time spent at the feeder of healthy cows (squares, n = 10) and those with SCK (diamonds, n = 10) during the transition period.....39

## LIST OF ABBREVIATIONS

**BCS** = body condition score

**BW** = body weight

**DM** = dry matter

**DMI** = dry matter intake

**FA** = fatty acids

**GH** = growth hormone

**NEB** = negative energy balance

**NEFA** = non-esterified fatty acid

**SCC** = somatic cell count

**SCK** = subclinical ketosis

**SE** = standard error of the difference between the means

**TMR** = total mixed ration

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## CHAPTER 1: INTRODUCTION

In 2007, milk products represented 87% of Canadian dairy farmers income and 13% of all farm income generated in Canada (CDIC, 2009c). Clearly, the production of milk is a major contributor to the viability of Canadian agriculture and especially important to the dairy industry. The amount of milk that a single farm can produce is based on a variety of factors including disease, plane of nutrition, number of animals in the herd and the production level of the individual animals. From 2001 to 2006 the number of farms that had dairy cattle decreased by 20% and the overall number of dairy cows decreased by 13%, yet the level of milk production remained stable (Statistics Canada, 2006). The stability of milk production is attributed to genetic selection for higher milk production, as well as improved understanding of the nutritional requirements of milk production (Rauw et al., 1998). For example, Holstein-Freisan cattle, which comprise 95% of the Canadian dairy herd (CDIC, 2009c), have been successfully selected for high milk production as indicated by the average milk production per cow increasing from 7,625 kg in 1990 to over 9,800 kg in 2008 (CDIC, 2009b).

Although culling due to low milk production has declined by 10% over the past decade, culling due to illness has remained stable, with approximately 37% of all culled dairy cows removed due to health issues such as mastitis, lameness or general illness (CDIC, 2009a). Disease not only affects the dairy industry through culling of potentially productive animals, but also through the association between disease and reduced milk production (Wallace et al., 1996; Fourichon et al., 1999). The stability of the high rate of culling due to disease indicates that, despite improvements in genetic selection and nutritional understanding, practical management of disease remains a major challenge in the dairy industry.

Disease management for dairy cows can be either reactive or preventative. Reactive approaches rely on monitoring animals for signs of disease, diagnosing the disease and reacting with corrective treatment. For example, clinical hypocalcaemia (milk fever) has been published: Ormandy E, Schuppli CA and Weary DM (2009) Worldwide Trends in the Use of Animals in Research: The Contribution of Genetically Modified Animal Models, Alternatives to Laboratory Animals 37: 63-68. It was co-authored by CA Schuppli and DM Weary, both supervisory committee members. These co-authors acted in the role typical of supervisory members. The main ideas for the manuscript were developed and researched by Elisabeth H. Ormandy. Co-authors supervised, helped interpret material and edited drafts. mia (milk fever) can be recognized by visual signs including hard dry feces, cold ears, lack of appetite and recumbancy (Radostits et al., 1994). Following diagnosis of these signs, animal caregivers (including dairy producers and veterinarians) are able to react by treating the ill animal with supplemental calcium (Ca) in hopes of correcting the low Ca levels that caused the symptoms. Unfortunately, many diseased animals fail to present distinct clinical signs. These animals are commonly referred to as suffering from subclinical disease, and are unlikely to be identified through traditional clinical signs used in reactive approaches. Clearly, the gold standard would be to implement preventative approaches, thereby preventing the disease from developing in the first place. The use of preventative approaches requires understanding of 1) when the disease is most likely to occur, 2) what factors increase the risk of disease, 3) what measures are most effective in reducing the risk of disease and, 4) practical control methods. Most research has focused on improved nutrition as a means of preventing clinical and subclinical disease. For example, milk fever occurs most often during the period immediately after calving. The risk of milk fever can be reduced, however, if the dietary cation-anion difference of the pre calving diet is controlled within a certain range (De Garis and Lean, 2008).

Clearly, disease management focusing on prevention is more beneficial by reducing costly treatments, loss of milk and involuntary culling. A benefit to both the producer and the cow is that disease prevention favours optimal biological functioning, which is a component of providing animals with good welfare (Duncan and Fraser, 1997; Fraser et al., 1997). Although efficient reactive approaches will always be required to treat clinically ill animals, good preventative approaches will reduce reliance on reactive methods and likely contribute to an overall increase in the welfare of dairy cattle. It is therefore essential to identify the risk factors for diseases if practical prevention strategies are to be developed.

It is well recognized that the highest concentration of disease during the adult life of a dairy cow is during the period after calving, the transition period, referring to the 3 wks before calving until the 3 wks after calving (Grummer, 1993). Cows experience numerous physiological (e.g. parturition, lactation) and managerial changes (e.g. regrouping, dietary changes) during the transition period. Most notable, however, is the increase in energy demands and concurrent decrease in energy intake. The imbalance in energy status results in most dairy cows experiencing a period of negative energy balance (NEB) after calving, which increases the risk of both metabolic and infectious diseases (Duffield, 2000).

The purpose of this review is to outline the current understanding regarding the relationship between feeding behaviours and the risk of disease during the transition period. This thesis will first briefly review the changes and challenges of the transition period, highlighting the changes in dairy cow physiology and group management. Secondly, it will review the nutritional approaches taken to reduce NEB, identify animal and human sources contributing to variation in dry matter intake (DMI) and, lastly, describe the etiology of ketosis, a disease directly related to NEB.

## **The Transition Period: Changes and Challenges**

### *Changes at Transition: Physiology*

The transition period involves a cow “transitioning” from a non-lactating state in late pregnancy to a lactating state following the birth of a calf. Most of these changes are coordinated by hormonal and metabolic adaptations, which are not mutually exclusive. These changes influence a cow’s nutritional requirements and, through interactions with behaviour and management, influence a cow’s ability to avoid disease. Although the changes in energy requirements, metabolism and physiology are not fully understood, the following brief review of the literature in this area provides clear evidence of why the transition period is so challenging for the modern dairy cow.

During the last 2 months of gestation, fetal growth becomes exponential (Bauman and Currie, 1980) and fetal metabolic rate is approximately two times the rate of the dam (Reynolds et al., 1986). The majority of fetal energy is derived from maternal sources, with 50-70% of fetal energy derived from glucose (Bauman and Currie, 1980; Bell, 1995). As glucose is provided to the fetus primarily through facilitated diffusion (Stacey et al., 1978), maternal adaptations are required to accommodate the increase in glucose demands from the dam’s system. These adaptations include increased hepatic gluconeogenesis, reduced use of glucose by peripheral tissues, increased mobilization of non-esterified fatty acids (NEFA) from adipose and increased use of NEFAs by peripheral tissues (Bell, 1995).

Mammary development parallels the development of the fetus, especially during late gestation (Thatcher et al., 1980). The development of the mammary gland, also known as mammogenesis, and the onset of lactation, known as lactogenesis, are influenced by signals originating from the fetus and placenta in anticipation of the calf’s needs after birth (Delouis et al., 1980; Bauman and Currie, 1980; Thatcher et al., 1980). Milk components, such as lactose

and fatty acids, appear in the mammary gland as early as 30 days prior to parturition (Hartmann, 1973; Mellenberger et al., 1973). Many of the adaptations to fetal demands also support the demands of the mammary gland via repartitioning of nutrients. For example, the increase in gluconeogenesis and mobilization of NEFA from adipose tissues provides energy and nutrients for the fetus as well as the mammary gland. Other metabolic changes in nutrient partitioning include mobilization of protein stores and increased absorption of minerals, most notably Ca (Bauman and Currie, 1980).

The hormonal and metabolic changes during the late gestation period that coordinate the growth and development of the fetus and mammary gland likely influence energy balance within the cow and the cow's ability to cope with post partum NEB (Grummer, 1995). For instance, there are many changes in levels of sex hormones around parturition (Chew et al., 1979; Squires, 2003). Some authors have hypothesized that these changes, specifically the elevation in estrogens before parturition, may contribute to the decline in DMI seen during the days prior to parturition (Grummer et al., 1990). More directly related to energy balance are the metabolic hormone changes, such as insulin and growth hormone (GH). Changes in these hormones have been identified as factors contributing to the control of energy balance in dairy cows during the transition period (Block et al., 2001). During the transition from late gestation to early lactation, insulin levels decrease and GH levels increase (Kunz et al., 1985). The changes in insulin and GH create a metabolic state that favours catabolism of maternal bodily energy stores, increasing the amount of energy available for the fetus and mammary gland.

#### *Challenges at Transition: Management*

In response to the physiological changes of the transition period, dairy cows also undergo numerous managerial changes, which may also affect behaviour. During the 3 wks before

calving, most dairy cows are regrouped into a “close-up” group as they approach their predicted calving date. In the close-up group, cows are fed a diet designed to prepare them for the upcoming calving event, as well as the demands of lactation. Cows then calve, often in an isolated maternity pen, sometimes requiring human assistance due to a malpositioned calf, twins competing for the birth canal or discrepancy between calf size and the size of the birth canal. Following parturition cows are again subjected to another regrouping into the lactating herd and milked 2-3 times a day. Some farms may require cows to go through additional regrouping. For example, they may have a non-saleable milk group, containing cows with milk that cannot be sold for human consumption (i.e. colostrum, milk from cows receiving antibiotics etc.). Cows that have just calved usually remain in this group for approximately six milkings and are then regrouped into the saleable milking herd.

As mentioned previously, the grouping strategies during the transition period reflect the changes in the needs of cows and the producers. Separating animals that are not producing milk from those which are facilitates the milking process and allows for better management of the nutritional needs of the cattle, which are dependent on stage of production. Research that has modeled the economic efficiency of different grouping strategies has shown that grouping cows based on nutrient requirements is the most effective way of minimizing the costs of milk production when compared to methods based on dry matter intake (DMI), days in milk (DIM) and genetic merit (Williams and Oltenacu, 1992). Grouping cows based on their nutrient requirements also allows for more accuracy between the formulated ration and an individual animal’s nutrient requirements (Nocek et al., 1985). Accurately meeting nutritional requirements is especially important during the transition period when increasing energy requirements and physiological changes are challenging the provision of optimal nutrition.

## **Coping with Change: Energy Management during the Transition Period**

The grouping of dairy cows during the transition period reflects an attempt to better meet the nutritional needs during late gestation and early lactation. The main focus of nutritional strategies is to prevent disease while supporting high levels of milk production. Management of transition diets has focused mainly on maximizing energy intake and reducing reliance on energy stores in the body, mainly fat (Grummer, 1995; Overton et al., 2009). Despite this, most dairy cows experience a 30% decline in DMI during the last 3 wks of the transition period (Hayirili et al., 2002), when nutritional demands are increasing rapidly due to the growth of the fetus and mammary gland.

To combat the decline in intake and increase in energy requirements, most transition diets are formulated to have high amounts of energy providing substrates. For the ruminant dairy cow, the main source of dietary energy comes from the digestion of carbohydrates into volatile fatty acids (VFAs), mainly acetate, propionate and butyrate (Schmidt et al., 1988). Although VFAs can provide energy to cattle, propionate is particularly important as it is converted to glucose in the liver and thus plays a pivotal role in regulating the production of alternative energy sources.

Ensuring high amounts of VFAs in transition diets is usually achieved by adding high levels of readily fermentable carbohydrates, also referred to as “concentrates” to the feed. One study reported that feeding high levels of concentrates during the prepartum period increased the length of the rumen papillae, allowing for increased absorption of VFAs (Dirksen et al., 1985), likely improving the energy status of the cow. This feeding strategy also allows for the rumen microbial system to adapt to the digestion of high concentrate feedstuffs, which is a large component of the post partum diet (NRC, 2001). Increasing the amount of concentrates in the prepartum diet can also increase prepartum insulin (Holtenius et al., 2003), which is typically

low during late gestation, potentially reducing the amount of fat mobilized and decreasing the health risks associated with excessive fat mobilization (Grummer, 1995).

Providing dairy cows with extra energy during the prepartum period has been shown to be a successful way to reduce the severity of NEB during the transition period. Administering supplemental propylene glycol (a direct source of glucose), during the prepartum period increases glucose levels and reduces signs of NEB (Studer et al., 1993). Improving the accessibility of energy from feedstuffs, via addition of ionophores to the diet, also reduces the severity of NEB during the transition period and reduces the mobilization of body lipids (Duffield et al., 2008; Zaraha et al., 2006). Perhaps the most convincing evidence that increasing energy supplied from the diet improves energy status in the transition cow is that provided by the experiment of Bertics et al. (1992). In this study, feed refusals were force-fed via a rumen cannula during the prepartum period, allowing for a consistent feed intake to be maintained. The consistent intake resulted in higher glucose and lower levels of liver triglyceride, indicating that there was more energy available for the cow to use.

### **Sources of Variation in Feed Intake**

Despite the use of nutritional management strategies aimed at optimizing nutrient composition of transition dairy cow diets, variation remains between individual cow's nutrient consumption. During the final 3 weeks of gestation, 19.7% of the variation in DMI was attributed to variance in animal factors, such as body condition score (BCS) (Hayirli et al., 2002). Recent research has highlighted that cow behaviour can have a major influence on DMI and the quality of the consumed diet. In addition to characteristics of the individual animal, management practices can affect the level of nutrition that individuals receive, and both likely influence the susceptibility to NEB during the transition period.



### *Animal Factors Influencing Variability*

The act of feeding begins with selecting substrates for consumption. Diet selection is based on gustatory, olfactory and tactile responses to the characteristics of feed (Phillips, 2002). For example, the palatability of a diet, often used synonymously with dietary preference, results from an interaction between these senses and post-ingestive feedback (Blundell and Rogers, 1991; Forbes, 1995). Of the four primary tastes (sweet, salty, bitter and sour; Jacobs et al., 1978), dairy cows have shown to preferentially ingest sweet-flavoured feeds (Nombekela et al., 1994; Broderick et al., 2008). Contrary to the findings of Nombekela et al. (1994) and Broderick et al. (2008), Ordway et al. (2002) found that there was no difference in intake when using the same sweetener with transition cows. One explanation for the discrepancy between these studies could be that Nombekela et al. (1994) and Broderick et al. (2008) used animals that calved a minimum of 7 days before the start of the experiment, thus, it is possible that the metabolic requirements of calving, and thus post ingestive feedback of diet meeting nutritional needs, affected the results of Ordway et al. (2002). It is therefore likely that dietary preference is affected by the nutritional status of the animal.

Once a cow has decided that the feed is suitable for consumption, ingestion requires use of the lips, teeth and tongue to move feed into the oral cavity where mastication and salivation are the first steps in digestion. Mastication breaks down large particles into smaller, more digestible pieces, while salivation lubricates feed and is the first stage in chemical digestion. These behaviours are the basic mechanisms of consumption and interact with external factors, such as feed DM, particle size and forage composition, to mediate the quantity and quality of nutrition that dairy cows receive. For example, feeds with higher levels of neutral detergent fiber (NDF) are often associated with more mastication during eating (Beauchemin, 1991; Allen,

2000; Beauchemin et al., 2003), which increases the amount of time dedicated to mastication and reduces time available for feed intake. Greenwood and Demment (1988) showed that when feed intake increased, mastication decreased. Although this has not been shown in the literature, the opposite is likely true: when mastication increases, feed intake decreases. Therefore, depending on the composition of the diet being fed, the amount of mastication and salivation required influences the amount of feed that a dairy cow can ingest and the extent to which the feed is broken down in the oral cavity.

Physical characteristics of the feed are not only important for mastication and salivation, but are also important in determining the quality of the consumed ration. In component feeding systems, animals can consume large amounts of one part of the diet without consuming adequate amounts of another, which can have negative effects on rumen function (Coppock et al., 1981). Thus, diets are often fed as a total mixed ration (TMR), where dietary components are mixed together to provide animals with a more consistent ration throughout the day. By physically mixing the dietary components together, the composition of each bite of food is also thought to be more consistent. The act of mixing, however, results in a range of particle sizes that is dependent on the mechanical method used to mix the ration. Unfortunately, cows are able to sort through these different particle fractions and select certain components for consumption. Many studies have shown that under a variety of conditions, dairy cows will sort against long particles and for smaller particles (Kononoff et al., 2003; Leonardi and Armentano, 2007; Hosseinkhani et al., 2008), regardless of the ratio of forage to concentrate (DeVries et al., 2007). As the time since delivery of fresh feed lengthens the amount of sorting for small particles increases, leaving more fiber in the bunk (DeVries et al., 2005; Hosseinkhani et al., 2008). Modern rations are formulated to meet nutritional needs by balancing the proportions of different components (Tisch, 2006) such that the shortfalls in one component are compensated by inclusion of another

complementary component (Coppock et al., 1974). The effect of sorting, in that it changes the relative proportion of different components in the consumed ration, causes imbalance in nutrient levels such that quality of feed available may differ from the balance ration and likely decreases throughout the day (DeVries et al., 2007).

### *Management Factors Influencing Variability*

As mentioned previously, cows are frequently regrouped as their needs change during the transition period. In feral cattle, however, group changes rarely occur and members form long lasting relationships between individuals (Lazo, 1994). The regrouping that occurs in commercial dairy farms disrupts these social relationships and elevates aggressive interactions, particularly at the feed bunk immediately after feeding (von Keyserlingk et al., 2008).

Immediately after entering a new group, the new cow experiences an increase in aggressive interactions, a reduction in feeding time and a decline in milk production (von Keyserlingk et al., 2008). Primiparous animals may not be familiar with the changes in social structure associated with regrouping (Phillips, 2002) and can benefit in terms of increased DMI and milk production when separated from multiparous cows (Phelps, 1992).

An increase in competition at the feed bunk is not only seen after changes in group composition, but can also result from managerial decisions about barn design and stocking density. Many studies have shown that decreasing available space at the feed bunk decreases time spent feeding and increases competitive behaviour (Olofsson and Wiktorsson, 2001; DeVries et al., 2004; Huzzey et al., 2006). The greatest increase in competitive behaviour occurs during the peak in feeding activity seen after delivery of fresh feed (DeVries et al., 2004). Since subordinate dairy cows will choose to eat away from dominant animals and will sacrifice feed

quality in exchange for larger distances from dominant cows (Rioja-Lang et al., 2009), dominant animals often have greater access to fresh feed and subordinate animals are forced to eat later (DeVries et al., 2004).

When physical protection against displacement is provided at the feed bunk, subordinate animals show the largest increase in feeding activity (DeVries and von Keyserlingk, 2006), likely due to being displaced less often (Huzzey et al., 2006; DeVries et al., 2006). If feed is provided more often or more feeding space is available per cow, displacement of subordinates from the feed bunk decreases and feeding time increases (DeVries et al., 2005; Huzzey et al., 2006).

The effect of competition on intake, however, is not as clear as the effects of competition on feeding time and displacements. Olofsson et al. (2001) found no difference in feed intake, despite a reduction in feeding time, when as many as four cows were competing for access to one feeder. Olofsson et al. (2001), however, fed finely chopped silage in a 50:50 ratio to concentrate, which may have permitted less mastication of the diet, allowing for more DMI. Furthermore, the design of the feeder in Olofsson et al. (2001) provided some protection against displacement from the feed bins, which may have influenced the affects of competition. Thus, the results of Olofsson et al. (2001) may not be applicable in situations when diets contain large amounts of forage or where no protection is provided at the feed bunk. In another study, using limited replicates and linear bunk space reduced to 0.2 m/cow, intake was not affected and there was little difference in time spent at the feed bunk (Friend et al., 1977). A more recent study found that during the transition period, rate was consistently increased by competition, but DMI was reduced only during the week before calving (Proudfoot et al., accepted). The increased energy requirements during the transition period may have influenced the cows' motivation to feed and reduced the affect of competition on DMI in the study by Proudfoot et al. (accepted).

Some dairy cows have been shown to engage in fewer displacements at the feed bunk during the week before calving, which was associated with the development of post partum uterine infections (Huzzey et al., 2007). More research is necessary to clarify the effects of competition on DMI during the transition period, but it does appear as though there is a relationship between competition at the feed bunk and poor post partum health.

From the aspects discussed here it is apparent that the feeding behaviours of dairy cows have a large impact on the quantity and quality of nutrition that an individual cow receives. The initial appraisal and apprehension of feeds influences quantity and quality of the consumed ration even when diets are fed as a TMR, as sorting behaviour allows dairy cows to select the components of the diet that they prefer to consume. Mastication and salivation act in concert with the physical characteristics of the diet to determine how long it takes a cow to consume a certain amount of DMI. This interaction becomes important when the time available for feed consumption is reduced, especially in competitive environments. During the transition period, the effect of feeding behaviour on DMI may influence our ability to formulate balanced rations that meet the rapidly changing nutritional requirements of the dairy cow, and thus affect the likelihood of disease in the post partum period.

### **Ketosis: When there just isn't enough Energy!**

As discussed in the previous sections, the changes and challenges faced by cows during the transition period create a major challenge for meeting the nutritional requirements of dairy cows during this period. To meet these challenges, the dietary concentration of energy is increased, however, group changes and feeding behaviour likely impact our ability to provide transition cows with adequate nutrition during the transition period.

When a cow's nutritional requirements are not met, the biological functioning of the dairy cow is at a higher risk of being disrupted, which increases the risk of both metabolic and infectious diseases (Mulligan and Doherty, 2008). Many factors contribute to the development of disease, such as genotype, environment and management. Diseases that are common to the transition period are hypocalcaemia, displaced abomasum, ketosis, mastitis, metritis and retained placenta (Mulligan and Doherty, 2008). Of these diseases, ketosis is directly related to the energy status of the cow.

As mentioned previously, propionate is an important energy substrate for ruminants as it is converted to glucose in the liver (Zammit, 1990; Hegardt, 1999; Vernon, 2005). When propionate is present in sufficient amounts to meet demands, pathways that derive energy from alternate sources are inhibited (Hegardt, 1999; Vernon, 2005). When propionate is not sufficient to meet gluconeogenic demands, such as when DMI is reduced or when glucose requirements exceed the amount produced from propionate, the inhibition of the production of alternative energy sources is reduced and the reliance on alternate energy substrates increases (Reynolds et al., 2003). Lipids, especially long chain fatty acids, become an important energy source as they are mobilized in response to poor energy status and are metabolized to ketones, an important source of energy which can be utilized by periphery tissues such as the heart, kidney, gastrointestinal tract, mammary gland and skeletal muscle (Heitmann et al., 1987).

Overproduction of ketones can be detrimental to the biological functioning of dairy cows (Duffield, 2000) and results in a condition known as ketosis (Andersson, 1988). One review found that prevalence of elevated ketones in the literature ranged from 8.9% to 34%, with the majority of cases occurring within the first two weeks after calving (Duffield, 2000). Although there is much debate as to what level of ketones indicates deviation from normal (Duffield, 2000), the extent to which ketones are elevated determines if this disease is clinically detectable

or exists as a subclinical condition. Clinical ketosis is characterized by depressed intake, hard dry feces, loss of body weight (BW), reduced milk production and, in severe cases, signs of nervous impairment (Radostits et al., 1994). In contrast, subclinical ketosis is hard to detect, as there are no overt signs, however, many tests are available to determine the presence of elevated ketone levels using bodily fluids such as urine, milk and blood (Geishauser et al., 2000). Many of these tests have poor performance and are only useful once the animal has already developed elevated ketone levels.

Many of the different classifications of ketosis reflect the fact that ketone production is generally elevated by reduced intake of energy, although it is possible to induce ketosis via overfeeding of highly ketogenic feeds (Kronfeld, 1982; Radiostitis et al., 1994; Duffield, 2000; Kaneko et al., 2008). Since most classes of ketosis are based on insufficient intake or intake of inadequate feedstuffs, it is likely that the feeding behaviours mentioned earlier that affect overall intake or consumption of sources of energy (e.g. sorting, competition) will have a large impact the risk of ketosis.

Restricting feed intake can be used to experimentally induce ketosis (Bahaa et al., 1997), but this does not imply that voluntary intake will be reduced in non-experimental cases. Recent research showed that voluntary feed intake, time spent feeding and feeding rate declined as early as four days prior to producer diagnosis of clinical ketosis (Gonzalez et al., 2008). The work of Gonzalez et al. (2008), however, did not specify the stage of lactation when animals were diagnosed with ketosis or if the decline in DMI was associated with a particular event, such as calving. As the largest incidence of both clinical and subclinical ketosis occurs during the period after calving (Duffield, 2000), more work is necessary to determine if the results of Gonzalez et al. (2008) are also applicable to dairy cows during the transition period.

## **Objective**

Although restricting intake can be used to induce ketosis (Bahaa et al., 1997), no research to date has connected voluntary DMI and the development of subclinical ketosis. Given that most cases of ketosis occurs during the 2 wks after calving, it is likely that prepartum voluntary DMI will be lower in dairy cows that develop post partum subclinical ketosis. Determining if there is a connection between voluntary DMI and subclinical ketosis will provide knowledge of the risk factors for subclinical ketosis that are necessary for developing preventative management programs. The objective of my research is to investigate feeding behaviour during the transition period of dairy cows that develop subclinical ketosis post partum and to determine if these behaviors differ from those of dairy cows who remain healthy during the transition period.

## **Hypothesis**

It was hypothesized that there would be a depression in feeding behaviours during the prepartum period for cows that developed subclinical ketosis during the week after calving. It was also hypothesized that subclinical ketosis during the week after calving would also be associated with more displacements from the feed bunk more often during the week before calving.



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## CHAPTER 2: PREPARTUM FEEDING BEHAVIOUR IS AN EARLY INDICATOR OF SUBCLINICAL KETOSIS<sup>1</sup>

### Introduction

During the transition period, defined as the 3 wks before and 3 wks after calving (Grummer, 1995), there is an increase in energy demands due to fetal growth and lactogenesis. Dairy cows often experience a decline in intake during the days before calving, which in combination with the increase in energy demands, results in a negative energy balance (NEB) during early lactation (Grummer, 1995; Drackley, 1999; Sovani et al., 2000). In an attempt to meet these increased energy demands cows can mobilize energy stores in their body (Bauman and Currie, 1980), such as fat, increasing the production of ketone bodies in the blood. Although elevated plasma ketone levels are normal around calving, abnormally elevated levels can result in clinical or subclinical ketosis (SCK).

About 30% of dairy cows experience SCK during the 2 wk after calving when energy demands for milk production are high (Duffield et al., 1998; Duffield, 2000). Both clinical and subclinical ketosis have been associated with increased risk of infectious disease and reduced reproductive performance (Reist et al., 2003; Walsh et al., 2007). However, most subclinical diseases go undiagnosed, making treatment difficult and prolonging any negative effects on herd health and productivity. Early identification of cows at risk of developing SCK could allow for timely intervention, potentially decreasing the negative effects of this disease.

Current tests for SCK are based on measuring ketone levels, mainly mainly  $\beta$ -hydroxybutyrate (BHBA), in the blood, milk and urine. Blood BHBA concentrations are currently the gold standard, but these tests are costly compared to cow-side tests (Duffield, 2000)

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and require time for laboratory analysis. A handheld electronic measuring device can provide a sensitive and specific cow-side test of BHBA levels in the blood of post partum cows (Iwerson et al., 2009), but has not been evaluated for use during the prepartum period. These tests, therefore, cannot identify at-risk animals prior to the onset of the disease.

Promising work in the area of early disease identification has focused on changes in feeding behavior as indicators for cows at risk of postpartum disease (Urton et al., 2005; Huzzey et al., 2007; Gonzalez et al., 2008). Huzzey et al. (2007), for instance, found that prepartum feeding behavior predicted the onset of clinical and subclinical metritis post-partum. In one study that examined cows diagnosed with clinical ketosis, Gonzalez et al. (2008) found that DMI decreased 3 d before clinical signs of the disease were observed. Other studies have successfully induced postpartum ketonemia by restricting post partum DMI and feeding ketogenic compounds (Drackley et al., 1992). However, no work to date has attempted to determine the predictive value of voluntary prepartum feeding behaviors on subclinical ketosis after calving.

Cows that develop metritis postpartum also engaged in fewer displacements at the feed bunk during the week before calving and avoid the feed bunk during periods when competition for feed is highest (Huzzey et al., 2007). DeVries et al. (2004) found that the effects of competition at the feed bunk were greatest for socially subordinate animals. The influence of social pressure on feeding behaviors during the pre-partum period may, therefore, influence susceptibility to post-partum disease.

The work of Huzzey et al. (2007) and Gonzalez et al. (2008) shows that feeding behavior can be used in the early identification of animals that later develop either infectious or metabolic diseases. Huzzey et al. (2007) also showed that social behavior at the feed bunk may differ between healthy and ill animals prior to the onset of illness. Thus the objectives of this study

were to investigate if feeding behaviors and social behavior during the transition period differed between animals that developed subclinical ketosis after calving and those that remained healthy.

## **Methods**

### *Animals, Housing and Diet*

Between August 2005 and March 2006, 32 primiparous and 69 multiparous (parity = 3.2  $\pm$  1.3; mean  $\pm$  SD) Holstein dairy cows were monitored. These are the same cows described in Huzzey et al. (2007), but the present study reports additional data that was not available at the time of that report. All animals were cared for according to the guidelines established by the Canadian Council on Animal Care (1993). Cows were housed in pre- and postpartum group pens, each maintained at 20 cows. The experimental pens provided 20 free-stalls, 12 electronic feeding stations and 2 electronic water stations (Insentec, Marknesse, Holland; see Chapinal et al., 2007 for a description and validation of this system). Cows entered the prepartum pen 25  $\pm$  2 d before expected calving date and were moved to the maternity pen when they showed 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 feeders and 1 Insentec water trough. Cows were moved to the postpartum pen within 24 h of calving and were monitored for an additional 21 d. After calving, cows were milked twice daily at approximately 0700 h and 1700 h.

Pre- and postpartum groups were fed a TMR at approximately 0800 and 1600 h. A detailed description of the nutrient analysis for each diet can be found in Huzzey et al. (2007).

### *Behavioural Data Collection*

Individual feeding behaviour was monitored using the Insentec electronic feeding system (Marknesse, Holland). Body weights (BW) were measured by averaging BWs taken on 3

consecutive days at entry into the trial, calving and 21 d postpartum. The same trained observers determined body condition score (BCS) at d  $-20 \pm 2$ , d  $-10 \pm 2$  and every 3 d after calving (1 to 5, following Wildman et al., 1982). Cows were identified as having retained placenta (RP) if the placenta was observed hanging from the vulva beyond 24 h after calving. Every 3 d after calving until +21 d, cows were subjected to a clinical examination to identify mastitis, metritis, milk fever, abomasal displacements and to measure rectal temperature using a digital thermometer (GLA M525/550, GLA Agricultural Electronics, San Luis Obispo, CA). Udder hardness, heat, and clots or discolored milk were used to identify clinical mastitis. Milk samples were taken at d +3, d +4, d +6, d +7, d +14, d +15, d +20 and d +21 and were analyzed for fat, protein, lactose and somatic cell count (SCC).

During health exams, blood samples were collected from the coccygeal vein using Vacutainer tubes (Vacutainer, Venous Blood Collection Tubes, BD Biosciences). Blood samples were allowed to clot for 3 h at room temperature and then centrifuged at 2500 RPM for 10 min. Serum was removed and stored at  $-20^{\circ}\text{C}$  until being sent to the University of Guelph Animal Health Laboratory (Ontario, Canada) for analysis of BHBA using a Hitachi 911 autoanalyzer (Roche Diagnostics, Hoffman-La Roche Ltd., Montreal, QC, Canada).

#### *Case Definition and Cow Participation in Study*

Due to the association between disease and feeding behavior during the transition period (e.g. Huzzey et al., 2007), animals diagnosed with any clinical diseases, including ketosis, RP or metritis, were excluded from the study.

Measurements of serum BHBA levels during the week after calving were used to classify animals as having SCK. A threshold of serum BHBA  $>1000 \mu\text{mol/L}$  in any sample taken on d 0, +3 or +6, was used to classify animals as having SCK based on the criteria used by Walsh et al.

(2007). Animals with BHBA  $\geq 1400$   $\mu\text{mol/L}$  in wk +2 or later were not included as this has been shown to be indicative of prolonged NEB and is often associated with clinical ketosis (Walsh et al., 2007). Cows with BHBA  $< 1000$   $\mu\text{mol/L}$  and no other disease were considered healthy.

Application of these criteria resulted in 9 multiparous cows and 1 primiparous cow diagnosed with SCK during the week after calving. One animal was identified on d 0 (BHBA = 1096  $\mu\text{mol/L}$ ), 8 on d +3 (BHBA ranging from 1083 to 2179  $\mu\text{mol/L}$ ) and 1 on d +6 (BHBA = 1071). Three of the 6 identified on d +3 also had elevated BHBA levels on d +6. These 10 animals with SCK were balanced for parity and calving difficulty with 10 healthy animals. The SCK and healthy groups had an average parity of  $1.8 \pm 0.4$  (mean  $\pm$  SE; range 1 to 4) and  $1.3 \pm 0.3$  (range 1 to 3), respectively. The average BCS before calving for animals with SCK and healthy animals was  $3.5 \pm 0.06$  and  $3.4 \pm 0.05$  (mean  $\pm$  SE), respectively. The average BCS after calving was  $3.3 \pm 0.05$  and  $3.3 \pm 0.05$  (mean  $\pm$  SE) for animals with SCK and healthy animals, respectively.

### *Social Behaviour Data Collection*

Two video cameras (CCTV camera, model WV-BP330; Panasonic, Osaka, Japan) were mounted over the feed bunk in both pre- and post-partum pens and continuously recorded behaviour throughout the experiment. Red lights (100W) were hung adjacent to the cameras to facilitate recording during low lighting (e.g. night time). Hair dye was used to mark the back and side of animals with alphanumeric symbols for easy identification of individuals. Displacements during the 3 h following the morning and afternoon feeding (peak feeding activity; DeVries et al., 2003) were recorded from 3 d selected randomly during the week before calving. An animal was recorded as the actor in a displacement when their head came in contact with an animal that was feeding, followed by the animal which was feeding removing their head

from the feed bin. An animal was recorded as a reactor when they were physically displaced from the feed bin. The actor was recorded as successful if they accessed the bin from which the reactor was removed. One animal was removed from the analysis of social behaviour for the SCK group due to missing video. The total number of times that an animal was the actor during an interaction was averaged over the 3 d of video providing a single value for each animal.

### *Statistical Analysis*

Complete data on feeding behaviour was available from 14 d before calving until 21 d after calving. Normality of the data was analyzed using PROC UNIVARIATE (SAS Institute, 2003) and 3% of the values were identified as extreme outliers (3 inter-quartile ranges beyond the first or third quartile of the dataset) and removed. Feeding data was summarized into five periods based on week relative to calving; wk -2 (d -14 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). The day of calving (d 0) was not included due to variation in the time and duration of calving.

Differences in feeding behaviour (DMI, feeding time, feeding rate, visits to the feeder) and water intake of healthy and SCK cows were analyzed using the PROC MIXED procedure by period (SAS Version 9.1, 2003). A visit to the feeder was defined as when the cow put her head into the INSENTEC bin until the point when she removed her head. Period was treated as a repeated measure with cow as the subject and a compound symmetry covariance matrix. Parity was not included in any model as groups were balanced for parity. To detect differences in DMI between SCK and healthy groups, cow was considered the experimental unit and the model included the fixed effects of health (SCK or healthy), weekly period and the interaction between health and weekly period. DMI was also considered as a percentage of BW, however, the results were not different from unadjusted values and thus only the unadjusted values are presented.

Preliminary analyses revealed that SCK cows were slightly heavier than healthy animals (average BW at wk -2 for healthy = 716.8 kg  $\pm$  24.03, SCK = 769.3kg  $\pm$  24.03) at the start of the trial, although there was no significant effect of BW at wk -2 on health ( $P = 0.14$ ) or on the change in intake from wk -2 to wk -1 ( $P = 0.3$ ). Thus BW is not discussed further.

To determine the predictive value of DMI and other variables (days on close up diet, gestation length, change in DMI from wk -2 to wk -1, pre- and post-calving BCS) on the risk of SCK, multivariable logistic regression was done using PROC LOGISTIC. Each variable was initially tested individually and variables with  $P < 0.1$  were retained in the final logistic model. Variables retained in the final model included DMI, feeding time, number of visits to the feeder during wk -1 and the change in intake from wk -2 to wk -1. Similar to Huzzey et al. (2007), increased intake during wk -1 was associated with increased time spent at the feeder ( $R^2 = 0.55$ ,  $P = 0.01$ ) and increased time spent at the feeder was associated with an increased number of visits to the feeder ( $R^2 = 0.53$ ,  $P = 0.02$ ). Also, higher DMI during wk -1 was associated with a greater change in intake from wk -2 to wk -1 ( $R^2 = 0.54$ ,  $P = 0.01$ ). Thus, the odds ratios reported are from separate models to avoid including highly related variables in the same model.

Average daily milk production was analyzed during 3 periods (d 4 to 21, d 22 to 60 and d 60 to 120 after calving). Differences in milk production between healthy and SCK groups were analyzed using a mixed model where period was treated as a repeated measure, cow as the subject and a compound symmetry covariance matrix. Analysis of fixed effects was carried out in the following order: health, period, health by period interaction. Composition of consecutive milk samples was averaged to provide one value for d +3 to +4, d +6 to +7, d +14 to +15 and d +20 to +21, resulting in 4 samples for each cow during d +3 to d +21. A mixed model was also used to analyze differences in milk composition between healthy and SCK groups using fixed effects entered in the following order: health, sample day and health by sample day. Sample was

treated as a repeated measure with cow as the subject. The interaction between health and sample day was significant ( $P= 0.05$ ) for the model testing the difference in lactose production, thus, the mixed model was run by sample day with health as a fixed effect.

Differences in the average number of times that an animal was an actor per day between health groups were tested using a t-test. Primiparous animals show different social behavior than multiparous animals during the transition period (Proudfoot et al., accepted), thus primiparous animals were not included in the analysis of social behavior.

## **Results**

### *DMI and Feeding Behaviour*

During wk -1, +1 and +2, DMI of cows with SCK was 18%, 26% and 20% lower than the DMI of cows that remained healthy after calving ( $P < 0.01$ , Figure 1A). These animals also visited the feeder 18%, 27%, 28% and 16% fewer times during wk -2, -1, +1 and +2, (Figure 1B), and spent less time at the feeder the same weeks (Figure 1C). There were no differences in the rate at which SCK or healthy animals consumed DM during the 5 wks of the trial (healthy =  $0.1 \pm 0.01$  kg DM/min, SCK =  $0.1 \pm 0.01$  kg DM/min,  $P = 0.91$ ). For both SCK and healthy animals, feeding rate increased by approximately 23% from wk -1 to wk +1. There was a trend for water intake of SCK cows to be lower during wk -2 and wk -1 (wk -2: healthy =  $45.2 \pm 4.2$  kg, SCK =  $36.7 \pm 4.2$  kg,  $P = 0.07$ ; wk -1: healthy =  $44.6 \pm 4.2$  kg, SCK =  $34.7 \pm 4.2$  kg,  $P = 0.03$ ). There were no differences in the time spent at the water bin (healthy =  $12.3 \pm 1.43$  min/d, SCK =  $12.3 \pm 1.43$  min/d,  $P = 0.99$ ) or number of visits to the water bin during any week in the trial (healthy =  $10.58 \pm 0.9$  visits/d, SCK =  $9.42 \pm 0.9$  visits/d,  $P = 0.35$ ).

### *Logistic Regression*

During the week before calving, DMI, visits to the feeder, feeding time and the change in average daily DMI from wk -2 to wk -1 were associated with an increased risk of developing SCK. The risk of a cow being diagnosed with SCK increased by 2.2 times for every 1 kg decrease in average daily DMI during wk -1 ( $P_{\text{Wald}}=0.02$ ,  $CI_{95}= 1.1, 4.3$ ). This model explained 49% of the variation in the risk of SCK ( $P_{\text{Likelihood Ratio}}<0.01$ ). The model for time spent at the feeder accounted for 57% of the variation in risk ( $P_{\text{Likelihood Ratio}}<0.001$ ), with the risk of SCK increasing by 1.9 times for every 10 min decrease in feeding time during wk -1 ( $P_{\text{Wald}}=0.01$ ,  $CI_{95}=1.15, 3.13$ ). When number of visits to the feeder during wk -1 was substituted for time spent at the feeder, the model accounted for 36% of the variation in risk ( $P_{\text{Likelihood Ratio}}=0.01$ ). Reducing the average number of visits during wk -1 by 10 increased the risk of SCK by 3.5 times ( $P_{\text{Wald}}=0.05$ ,  $CI_{95}=0.96, 12.34$ ). A 1 kg decrease in average daily DMI from wk -2 to wk -1 increased the risk of developing SCK by 4.0 times ( $P_{\text{Wald}}=0.03$ ,  $CI_{95}=1.14, 14.29$ ) and the model accounted for 51% of the variation in risk ( $P_{\text{Likelihood Ratio}}=0.002$ ).

### *Milk Production and Composition*

Average daily milk production during the first 21 d after calving did not differ between health groups (healthy =  $42.12 \pm 2.4$  kg, SCK =  $42.70 \pm 2.4$  kg,  $P=0.86$ ). There was also no difference in total milk production during any of the 3 periods tested. There was no difference in milk fat content between healthy cows and those with SCK (Table 2). Animals with SCK did, however, have a lower concentration of milk protein and lactose (Table 2) and there was a health by sample day interaction for lactose production. Animals with SCK produced less lactose in the first 2 milk samples (d 3 to 4 and d 6 to 7;  $P < 0.05$ ) and there was a trend ( $P < 0.1$ ) for lower lactose production in the 3<sup>rd</sup> milk sample taken during d 14 to 15 post partum (Table 2).



Differences in lactose production between healthy cows and those with SCK were no longer evident by d 20 to 21.

#### *Social Behaviour at the Feed Bunk*

The average number of times in wk -1 that a multiparous animal was the actor was higher for healthy cows versus those with SCK (healthy =  $14.4 \pm 1.83$  number of displacements as actor/d, SCK =  $10.2 \pm 0.62$  number of displacements as actor/d,  $P = 0.05$ ).

### **Discussion**

The reported incidence of SCK in lactating dairy cows is dependent on the sensitivity the methods used to detect ketones and the threshold values used to diagnose the disease. Using the same threshold as in our study, Walsh et al. (2007) reported a 36% incidence when 796 Holstein dairy cows tested on 25 farms in Ontario. Other studies have used a higher threshold than that used in the current study (1200-1400  $\mu\text{mol/L}$ ; Duffield, 2000), but more recent work suggests that reproductive performance is impaired when BHBA levels exceed 1000  $\mu\text{mol/L}$  during wk +1 (Walsh et al., 2007). Our observation that cows with SCK have altered feeding behaviour in the week before and after diagnosis further supports the  $\geq 1000$   $\mu\text{mol/L}$  threshold as being associated with a physiological imbalance, which is generally indicative of disease (Ingvarsten, 2006).

Reduced DMI is a well-recognized sign of clinical ketosis in dairy cattle (Duffield, 2000). Gonzalez et al. (2008) found that several days before the diagnosis of clinical ketosis, the fresh matter intake by sick animals was 10 kg/d lower than normal. In the current study, DMI was 3 kg/d lower in SCK animals than in their healthy counterparts during the week before diagnosis of SCK in wk +1. Considering that our diet was approximately 50% DM, the reduction

in intake during the week before calving (i.e. the week before diagnosis of SCK) was slightly smaller than that found by Gonzalez et al. (2008); this difference is not surprising given that the cows in the present study were diagnosed with SCK and not clinical ketosis. To our knowledge, this is the first study reporting declines in intake and feeding behavior in association with the event of calving for cows that were diagnosed with SCK during the week after calving.

Similarly to Huzzey et al. (2007), cows that were diagnosed as ill post partum in our study spent less time at the feed bunk, visited the feeder less often and consumed less DMI in the week before calving. Huzzey et al. (2007) report that a 1 kg decrease in DMI during wk -1 increased the risk of metritis by 2.87 times, which is similar to the 2.2 times increase in the risk of SCK found in our study. Huzzey et al. (2007) and Urton et al. (2005) reported that a 10 min decrease in average daily feeding time during the week before calving was associated with a 1.7 and 1.6 times increase in the risk of metritis, respectively, which is similar to the 1.9 fold increased risk of SCK in our study. We also found that every 1 kg decline in the change in average daily DMI from wk -2 to wk -1 was associated with a 4.0 times increase in the risk of SCK. The results of the current, study combined with the findings of Huzzey et al. (2007) and Urton et al. (2005), provide evidence that feeding behavior and DMI during the week before calving are important risk factors for post partum disease.

We also found that cows that developed SCK had lower water intake during the 2 wk pre partum. This result is expected given the relationship between DMI and water consumption (Murphy, 1992). Failure to see greater differences in water intake between healthy and SCK individuals may reflect the insensitivity of using changes in water intake to predict cows at risk for disease. Although Lukas et al., (2008) reported that water intake could be used as an alternative to monitoring DMI for disease identification; Huzzey et al. (2007) found that the risk of metritis associated with reduced water intake during wk -1 was much lower than the risk

associated with reduced DMI. We encourage future work in this area to verify these conflicting results.

For a variable to be useful in early identification of animals at risk of developing a disease it must be characteristic of the disease and precede diagnosis (Gonzalez et al., 2008). Given these criteria, the association between DMI, feeding behaviors and increased risk of uncomplicated SCK in wk +1 found in our study indicates that these variables are likely suitable for prepartum identification of animals at risk of developing post partum SCK. Although the current technology required to accurately monitor feeding behavior and individual intakes on commercial dairies is expensive, these systems provide objective measures that are readily accessible and useful in early identification of animals at-risk of developing postpartum SCK. More research is needed to establish values of feeding behaviours, such as DMI during wk-1 and change in average daily DMI from wk -2 to wk -1, that are cut-off values predictive of an increased risk of SCK. Additional research should focus on ways to incorporate these variables into practical on-farm disease monitoring systems.

SCK is believed to develop from the increasing energy requirements during the early post partum period. The lack of difference in milk yield between healthy and SCK animals, despite SCK animals having lower DMI, indicates that the requirements for milk production were met through energy sources other than feed. Since ketones are produced as a result of fat metabolism in the liver (Drackley et al., 2001), it is likely that body fat was used as an alternate energy source. Moreover, the lack of difference in milk fat provides evidence that BHBA was likely used for milk fat synthesis in the mammary gland of SCK animals (Akers, 2002). Despite the lack of difference in milk yield and milk fat, production of milk lactose and protein was different during the first 2 wk after calving. Although we did not measure serum glucose levels, reduced

production of milk lactose likely reflects a reduction in the amount of glucose available to the mammary gland, as it is the major precursor of mammary lactose (Akers, 2002).

Similar to the results of Huzzey et al. (2007), animals that were diagnosed as ill post partum engaged in fewer displacements at the feed bunk during peak feeding periods in the week before calving. As competitive social environments are known to alter feeding behaviour (e.g. Huzzey et al. 2006), reduced displacements at the feed bunk indicate that cows that became ill one week later were less motivated to compete for feed before calving. Proudfoot et al. (accepted) showed that when fed in a competitive environment, multiparous cows tended to have lower DMI during the prepartum period than cows that were fed in a non-competitive environment, likely increasing the risk of subclinical disease in competitively fed cows. The stocking density in our experiment was approximately 1.7 cows per feed bin; this is slightly lower than the 2:1 stocking density used by Proudfoot et al. (accepted) but likely resulted in competition at the feed bunk. Whether competition influenced the motivation to feed or visa versa is unclear. The results of this study and others (e.g. Huzzey et al. 2007; Proudfoot et al. accepted), however, provide evidence in support of the hypothesis that prepartum social interactions can influence postpartum health. More research is necessary, particularly with primiparous cows, to determine if there is a direct relationship between social status and post partum disease.

## **Conclusion**

Animals that developed SCK during the week after calving showed differences in feeding behaviour and DMI as early as 1 wk before calving. SCK animals also initiated fewer displacements at the feed bunk during the week before calving. The results of our study highlight the importance of implementing management practices that reduce competition and

increase feed bunk access, particularly in the prepartum period, as these are likely to be key factors in the prevention of diseases common to the transition period.

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**Table 1.** Least squares means ( $\pm$ SE) for milk composition of healthy cows (n=10) and SCK cows (n=10) during the 3 weeks following calving

		Sample Days Relative to Calving			
		+3-4	+6-7	+14-15	+20-21
Fat (%w/w)					
Healthy		5.69 $\pm$ 0.3	5.12 $\pm$ 0.5	4.86 $\pm$ 0.3	4.59 $\pm$ 0.2
SCK		6.20 $\pm$ 0.3	5.10 $\pm$ 0.5	4.55 $\pm$ 0.3	4.21 $\pm$ 0.2
Protein (%w/w) †					
Healthy		4.19 $\pm$ 0.1	3.80 $\pm$ 0.08	3.33 $\pm$ 0.07	3.33 $\pm$ 0.1
SCK		4.05 $\pm$ 0.1	3.58 $\pm$ 0.07	3.15 $\pm$ 0.06	3.02 $\pm$ 0.1
Lactose (%w/w)					
Healthy		4.23 $\pm$ 0.07	4.38 $\pm$ 0.07	4.61 $\pm$ 0.05	4.64 $\pm$ 0.05
SCK		3.93 $\pm$ 0.06**	4.15 $\pm$ 0.07*	4.48 $\pm$ 0.04†	4.56 $\pm$ 0.05

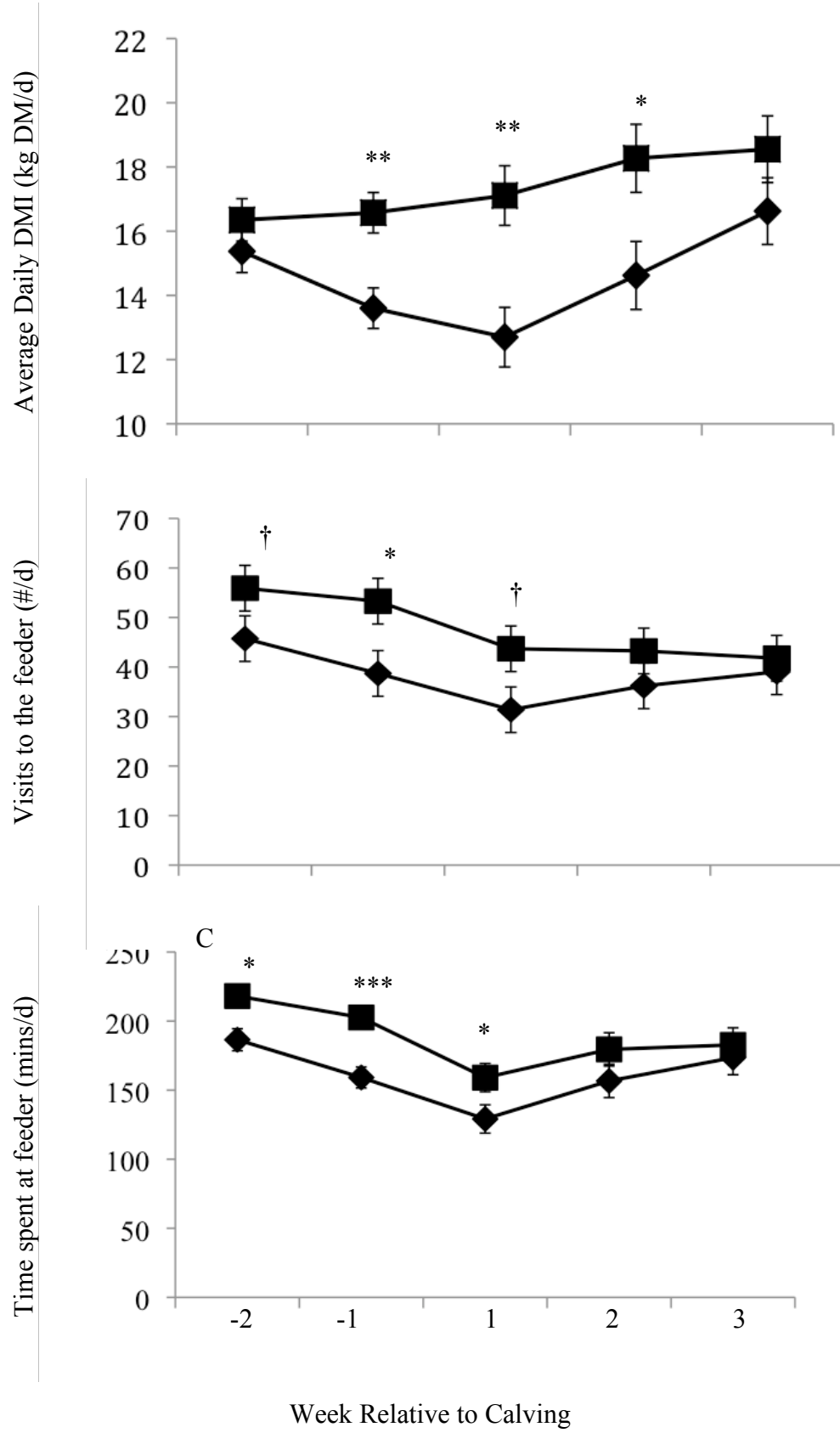
SCK = subclinical ketosis during the week after calving

Significance levels for difference between healthy and SCK: † P<0.1; \*P<0.05; \*\*P<0.01;

\*\*\*P<0.001

**Figure 1.** Least squares mean ( $\pm$ SE) for A) DMI, B) number of visits to the feeder and C) time spent at the feeder of healthy (squares, n = 10) and SCK (diamonds, n = 10) Holstein dairy cows during the transition period.

† P<0.1; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001





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### CHAPTER 3: GENERAL CONCLUSIONS

Animal welfare research strives to identify, solve and prevent welfare problems by investigating multiple components that can be grouped together based on a common function (i.e. contributing to the state of well-being that an animal experiences), but that cannot be ranked in any objective way (Fraser, 1995). In essence, overall welfare is more than a simple sum of its parts, but rather it includes the animal's subjective experience of the relationship between the components. Thus, maintaining good health and preventing poor health is a component of improving the welfare of animals, but health alone does not provide a measure of overall welfare (Fraser, 1997).

In the dairy industry there has been a paradigm shift from reactive treatments for disease to strategies for disease prevention (LeBlanc et al., 2006). Initially this shift involved heavy reliance on nutritional strategies for disease prevention. More recently, however, there has been a recognition that behavioural indicators of illness, often referred to as “sickness behaviours”, may be useful in identifying cows at higher risk of developing disease, especially during the transition period (Weary et al., 2009). As dairy cattle are prey animals and thus stoic in nature (Weary et al., 2009), the invention of automated technologies for recording behaviour, such as the INSENTEC feeding system used in the research of this thesis, has allowed for more sensitive measures of behaviour and contributed to the ability to detect behavioural changes associated with increased risk of illness.

During the transition period, feeding behaviours and managerial decisions that impact feeding behaviours have been shown to affect DMI in the period before calving (Proudfoot et al., accepted). Cows showing reduced DMI during the week before calving also have an increased risk of infectious uterine disease post partum (Urton et al. 2005; Huzzey et al., 2007). There is

little research, however, on the interaction between feeding behaviours and the risk of metabolic disease during the transition period.

Given the connection between nutrition and feeding behaviour and between nutrition and the risk of metabolic diseases, the purpose of this thesis was to investigate if a connection existed between feeding behaviour and the risk of metabolic disease, specifically ketosis, when the incidence was highest (i.e. the transition period). Other studies have used restricted intake to induce ketosis (Bahaa et al., 1997), however, induction of a disease fails to account for the development of disease under “natural” conditions (Weary et al., 2009). The experiment by Gonzalez et al. (2008) provided some evidence that feeding behaviours could identify dairy cows that developed clinical ketosis as early as 3 d prior to diagnosis by a producer. Gonzalez et al. (2008), however, failed to identify the stage of lactation of the experimental cows used in their study and did not investigate subclinical disease. Although early identification of clinical diseases is extremely important, it is often harder to diagnose subclinical disease let alone identify cows at risk of developing subclinical conditions.

The important finding of this study, therefore, was that voluntary DMI, feeding time and visits to the feeder declined in the week before calving in dairy cows that developed subclinical ketosis after calving. The magnitude of decline in DMI before calving was also associated with an increased risk of subclinical ketosis. These findings provide evidence in support of feeding behaviours as important risk factors associated with post partum disease and create a connection between prepartum voluntary feeding behaviour and post partum metabolic disease.

## **Future Research**

Urton et al. (2005) and Huzzey et al. (2007) were the first studies to show that feeding behaviours could serve as an early indicator of post partum infectious disease. The research undertaken in this thesis is the first to demonstrate that in a freestall environment, the feeding behaviours of dairy cattle are also early indicators of post partum metabolic disease and substantiates the use of feeding behaviours as an early indicator of illness.

The finding that dairy cows at highest risk for post partum metabolic disease engaged in fewer social interactions at the feed bunk before calving is similar to the findings of Huzzey et al. (2007), where cows at risk of post partum infectious disease engaged in fewer displacements at the feed bunk during the week before calving. Both the research in this thesis and the research by Huzzey et al. (2007) also found that cows at most risk of post partum disease had reduced DMI during the week before calving. Reduced DMI before calving has been shown to be associated with competition at the feed bunk (Proudfoot et al., accepted) and it has also been found that the feeding behaviours of subordinate cows are affected the most by competition (DeVries et al., 2004). Future work should investigate if there is a connection between competition and reduced DMI during the transition period and reduced DMI before calving and increased risk of disease after calving. It may also be of use to investigate the impact of social order on the nutrition of the consumed ration during the transition period and if effects of social order on feeding behaviour influence the risk of disease. Findings from such studies may lead to changes to ration formulation and barn design that reduce the risk of disease during the challenging transition period.

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