EFFECTS OF SAWDUST BEDDING DRY MATTER ON LYING BEHAVIOUR OF DAIRY COWS: A DOSE DEPENDENT RESPONSE

by

LINDSEY JANELLE REICH

B.Sc., The University of Delaware, 2008

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Animal Science)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April 2010

© Lindsey Janelle Reich, 2010

ABSTRACT

The objective of this thesis was to determine the effects of bedding dry matter on lying behaviour of Holstein cows. Over time bedding becomes wet with urine, feces and milk, but no research is available to guide recommendations for farmers regarding how often bedding should be replaced. I carried out two replicates of an experiment testing the effects of varying dry matter content of sawdust bedding systematically over five treatment levels. One replicate was conducted during the summer and one in the winter to test if the effects of damp bedding varied with season. The five bedding treatments averaged (\pm SD) 89.8 \pm 3.7, 74.2 \pm 6.4, 62.2 \pm 6.3, and 43.9 ± 4.0 , and 34.7 ± 3.8 % dry matter. Over the course of the trial, minimum and maximum temperatures in the barn were 2.6 \pm 2.0 and 6.8 \pm 2.2° C in the winter and 13.3 \pm 2.5 and 22.6 \pm 4.1° C in the summer. In both seasons, five groups of three non-lactating cows were housed in free stalls bedded with sawdust. Following a five day acclimation period on dry bedding, groups were exposed to the five bedding treatments in a five by five Latin square. Each treatment lasted four days, followed by one day when the cows were provided with dry bedding. Stall usage was assessed by 24 hour video scanned at five minute intervals averaged over two days. Responses were analyzed in a mixed model with group as the observational unit. Bedding dry matter affected lying time, averaging 10.4 ± 0.4 hours per day on the wettest treatment and increasing to 11.5 ± 0.4 hours per day on the driest bedding. Lying time varied with season, averaging $12.1 \pm$ 0.4 hours per day across treatments during the winter and 9.9 ± 0.6 hours per day during the summer, but season and bedding dry matter did not interact. These results show that wet bedding reduces lying time in a dose dependent manner during both winter and summer seasons.

TABLE	OF	CONTENTS
-------	----	----------

ABSTRACT	ii
TABLE OF CONTENTS	iii
LIST OF FIGURES	v
ACKNOWLEDGEMENTS	vi
CO-AUTHORSHIP STATEMENT	vii
CHAPTER 1: GENERAL INTRODUCTION	1
DAIRY INDUSTRY TODAY	
ANIMAL WELFARE SCIENCE.	
Lying Behaviour and Dairy Cows	
STALL DESIGN AND STALL USE	
Lameness	5
Mastitis	
EFFECTS OF MANAGEMENT ON LYING TIME	
Overstocking	
Quality of the Lying Surface EFFECTS OF ENVIRONMENT ON LYING TIME	
LYING BEHAVIOUR.	
LYING BEHAVIOUR	
Lying Bouts	
Resting Behaviour	
Lying Time and Bedding Dry Matter	
OBJECTIVES	
Hypothesis	
References	
CHAPTER 2: EFFECTS OF SAWDUST BEDDING DRY MATTER ON LYING	G
BEHAVIOUR OF DAIRY COWS: A DOSE DEPENDENT RESPONSE	
INTRODUCTION	
MATERIALS AND METHODS	
Animals, Housing and Diet	
Treatments and Experimental Design	
Behaviour	
Temperature Statistical Analysis	
RESULTS	
Bedding Dry Matter	
Season	
DISCUSSION	
Bedding Dry Matter	
Effect of Season	

References	31
CHAPTER 3: CONCLUSIONS	
CONTRIBUTIONS	33
FUTURE RESEARCH	
SUMMARY	
References	40
APPENDIX 1: BEHAVIOURAL MEASURES	
APPENDIX 2: BEHAVIOURAL MEASURES BY SEASON	
APPENDIX 3: LINEAR REGRESSION FOR SEASONAL EFFECTS	
APPENDIX 4: ANIMAL CARE CERTIFICATE	

LIST OF FIGURES

- **FIGURE 2.1.** TIME (HOURS/DAY) SPENT A) LYING IN THE STALL, B) STANDING IN THE ALLEY, AND C) FEEDING, SHOWN SEPARATELY FOR SUMMER AND WINTER REPLICATES. DATA WERE AVERAGED FROM FIVE GROUPS, FOR TWO DAYS ON EACH TREATMENT USING A LATIN-SQUARE DESIGN. 30

ACKNOWLEDGEMENTS

First, I would like to thank my committee members for their guidance throughout my experience with the Animal Welfare Program. Nina von Keyserlingk's enthusiasm, in addition to always making time for me no matter what, has helped me to not regret moving outside of my comfort zone. I also thank Dan Weary for his challenging yet ultimately effective teaching methods and Doug Veira for his assistance and support during my time in Agassiz. Thanks also to David Fraser for making lunch and class the highlights of my days at UBC.

I would also like to acknowledge Katherine Hume for her unexpected gift towards funding my education. Her support of my research as a result of her interest in animal welfare science is greatly appreciated.

My research would not have been possible without the assistance of Elsie Dawn Parsons, Lori Vickers, Joao Costas, Pilar Sepulveda, and everyone else who ever shared the joy of mixing and changing sawdust with me. I also appreciate Nelson Dinn's tolerance and understanding that made my trials possible, in addition to the endless help I received from all of the farmers in Agassiz.

I thank Nuria Chapinal for her enthusiasm and help with SAS and Kiyomi Ito for providing useful comments on my thesis. Thanks also to Katy Proudfoot for looking over anything I ever sent to her and for always having a calm and encouraging attitude about research. I would like to thank all of the friends I have made through the Animal Welfare Program – at the farm and in Vancouver. Thanks especially to Kathrin Schirmann for joining me for many bowls of pasta and reminding me to take breaks.

My general welfare, in Delaware and all the way in Vancouver, has greatly benefitted from my friendship with Megan Hofherr. I am so grateful for having a friend, despite being far away, with whom I could share this graduate school experience.

I would also like to thank the Hartman family for allowing a small and inexperienced eleven year-old the opportunity to fulfill her childhood dream of working on a dairy farm. Without those seven years of feeding calves and learning about cows, I doubt I would ever have become involved in the dairy industry.

Thanks also to Jessica and Anthony Madeira – to Tony for pointing out that spending many weeks on end watching videos of cows does seem a bit strange, and to Jess for being the best and most supportive sister I could ever want. Most importantly, I thank my parents for always supporting me throughout my education and in every aspect of my life. From my early dream of becoming a farmer to my later dream of studying dairy cow welfare, their understanding and encouragement has made pursuing any goal possible.

Finally, many thanks to Lucas Gebhart for the never ending support, patience, encouragement, even more patience, and proofreading during my Master's degree. The hard work and determination he has demonstrated has provided me with much motivation, and I am fairly certain this process would have been much more difficult without him.

CO-AUTHORSHIP STATEMENT

Lindsey Reich and Drs. Dan Weary, Doug Veira and Marina von Keyserlingk designed the study collaboratively. Lindsey Reich carried out the experiment, analyzed the data, and wrote the manuscript under the supervision of Drs. Dan Weary, Doug Veira and Marina von Keyserlingk.

CHAPTER 1: General Introduction

Dairy Industry Today

In 2007, milk ranked third in terms of agricultural commodities in Canada and first in the United States (FAOStat, 2010). Canada's milking herd is made up of about 982,800 milking cows (Statistics Canada, 2009) while in 2007 the United States had just over nine million milking cows (USDA, 2007). These cows produced around eight million and 84 million metric tons of milk in Canada and the United States, respectively (FAOStat, 2009).

Over the last 50 years, food animal production systems have become highly mechanized which enabled intensification of animal production. This gave rise to indoor facilities where animals are rarely provided access to the outdoors (Fraser, 2008). For example, chickens have been moved from outdoor "free range" housing systems to indoor battery cages. Similarly, few pigs are provided access to the outdoors and are instead confined sometimes in gestation and farrowing crates. Dairy systems sometimes include the use of pasture, but a large proportion of herds are housed continuously indoors (i.e. zero grazing).

Animal Welfare Science

One important concern regarding animal welfare is whether the animal is able to live a reasonably natural life, engaging in those natural behaviours that it is highly motivated to perform (Fraser, 2008). The passing of California's 2008 Proposition Two

ballot initiative¹, requiring that farm animals should be able to move freely, provides an example of the public's concern over the confinement of animals in agriculture. More generally, the growth of welfare labeling systems, such as the "BC SPCA Certified" labels on eggs and other agricultural products, suggests that consumers are searching for reassurance that these products are coming from animals that have been treated humanely.

Different groups of stakeholders have varying views of animal welfare (Fraser et al., 1997). For example, producers may be especially concerned about the health of their animals and the effects of poor health on production. Consumers, however, may be more concerned about how the animals feel and if they are able to live natural lives and exhibit natural behaviours. Fraser et al. (1997) described animal welfare science as a threefold concept, comprised of: 1) the affective state or an animal's ability to experience positive and negative emotions, 2) the biological functioning of the animal, and 3) the ability to exhibit behaviours as the animal would in its natural environment.

Assessment of welfare requires consideration for all three areas. Clearly, maintenance of biological functioning and good health must be a concern. For example, lame cows often show reduced milk production (biological functioning; Green et al., 2002). Lameness is also painful (affective state), and lame cows have difficulty showing normal reproductive behaviour (natural behaviour; Walker et al., 2008). Welfare scientists try to optimize all three aspects of animal welfare, although any one specific experiment may focus on just a single aspect.

¹ In the United States, citizens of 24 states and the District of Columbia can collect signatures for a change to their state's legislation and have the general public vote on the issue in question. If the general public votes to pass the proposed initiative it becomes law (US History Encyclopedia, 2010).

Lying Behaviour and Dairy Cows

Most dairy cows are housed indoors in tie stall or free stall barns. In tie stall systems, cows are restricted to individual stalls where they are fed individually and are either milked directly in the stall or walked to a milking parlour. In free stall systems, cows are housed in groups, have a number of stalls to choose from for lying, and feed from a common feed bunk.

Producers are faced with numerous decisions regarding how to design and manage these facilities. These decisions have typically been made with little or no scientific basis, in part because little information is available. However, there is now a growing body of research on the effects of stall design and maintenance on cow behaviour, including the amount of time cows spend using stalls for lying or standing.

Cows spend about half of their time lying down (Metz, 1985; Dechamps et al., 1989; Haley et al., 2000) and are motivated to maintain this lying time (Jensen et al., 2004; Jensen et al., 2005). Several studies have shown that lying time is valuable to dairy cattle, and when prevented from lying cows will "work" to lie down in order to maintain lying time (Jensen et al., 2004; Jensen et al., 2005). Munksgaard et al. (2005) found that cows spent the highest amount of time regaining lost lying time following restriction from lying, feeding, and social contact. This finding suggests that cows are motivated to maintain lying time even if it results in decreased time spent on other behaviours. Cows compensated for lost feeding time by increasing their feeding rate (Munksgaard et al., 2005), but this is not possible for lying. Dairy cows exhibit behaviours such as swinging their heads, stamping their feet, and repositioning their bodies when deprived of lying for even short periods of time (Cooper et al., 2007).

Stall Design and Stall Use

Time spent lying is dependent on the design of the lying area. Dairy cattle housing systems are designed to keep animals healthy, but at a minimum in cost and with minimal labour to keep cows and stalls clean. These contrasting goals have resulted in the use of restrictive hardware that can negatively affect stall usage, including lying times (Tucker et al., 2004; Tucker et al., 2006) and standing times in the stalls (Tucker et al., 2005; Bernardi et al., 2009; Fregonesi et al., 2009). Various hardware elements are intended to keep stalls clean by restricting cows from lying too far forward or standing in the stalls and urinating and defecating on the stall surface. Hardware used in the stall includes stall partitions, the brisket board, and the neck rail (Figure 1.1).

Tucker et al. (2004) reported how dairy cattle increased lying times by one hour per day when provided wide stalls compared to narrower stalls, possibly due to reduced contact with stall partitions. Furthermore, the brisket board, which indexes the cow within the stall and prevents her from lying too far forward in the stall, can reduce lying time by one hour per day (Tucker et al., 2006).

Similarly, several studies have shown that the placement of the neck rail affects stall use. Neck rails placed in very restrictive positions reduce the amount of time cows spend standing fully in the stalls (Tucker et al., 2005; Bernardi et al., 2009; Fregonesi et al., 2009). Standing in the stalls can be preferable for cows kept in intensive free stall systems because the stall surface provides a softer and drier place for standing than the alleys (Tucker et al., 2005). Bernardi et al. (2009) demonstrated that increased time spent standing fully in the stalls resulted in reduced lameness, suggesting that less restrictive

neck rails are most beneficial for lame cows. Thus despite any benefits associated with improved stall and cow cleanliness, producers must keep in mind that restrictive stall designs can reduce lying times, reducing the health of cows and increasing injuries. *Lameness*

Lameness is expensive for dairy producers (Esslemont and Kossaibati, 1996), and has been associated with decreasing milk production by about 360 kg per lactation (Green et al., 2002), poor reproductive performance such as increased calving interval (Sprecher et al., 1997; Sogstad et al., 2006), and increased involuntary culling from the herd (Booth et al., 2004; Bicalho et al., 2007). Moreover, lameness causes pain, reducing welfare for the dairy cow (Webster, 1986).

Increased standing time has been associated with increased incidences of lameness (Galindo and Broom, 2000). Additionally, lame cows change their lying behaviour and may spend more time lying down to relieve pain experienced while standing (Juarez et al., 2003, Chapinal et al., 2009). Lame animals have been shown to spend over four hours per day more time standing in the free stall than sound cows when housed on mattress stalls (Cook et al., 2004). Therefore, reducing standing time in the stalls, for example as a result of restrictive neck rails, could potentially cause cows pain, especially in lame cows because these animals would be forced to spend more time standing on hard alley surfaces rather than in stalls.

Mastitis

Aspects of stall design can have other effects on biological functioning. As described above, hardware elements such as the stall dividers, brisket boards, and neck rails, are used to keep manure in the alley rather than on the stall surface. Manure in the

stalls could aide in bacterial growth on the stall surface (Zehner et al., 1986). Although wider stalls have been associated with increased lying time they have also been associated with decreased stall cleanliness; more fecal matter was present on the stall surfaces of wide stalls compared to narrower stalls (Tucker et al. 2004). This decrease in cleanliness has been attributed to increased lying and standing with all four feet in the stalls. This is likely a consequence of cows being able to stand and lie diagonally in the wide stalls and an increased occurrence of cows urinating and defecating on the stall surface (Tucker et al., 2004). Furthermore, while the direct effect of brisket boards on stall cleanliness has not been thoroughly examined, Tucker et al. (2006) reported that stalls become soiled when cows defecate while lying down more often than when standing up in the stall. Bacterial counts on the stall surface are associated with bacterial counts on teat ends of cows, potentially increasing the risk of mastitis (Rendos et al., 1975; Hogan et al., 1999; Zdanowicz et al., 2004).

Fregonesi et al. (2009) recorded stall and udder cleanliness when cows were tested with neck rails placed at varying distances from the back end of the stall to restrict how far forward cows can stand in the stall. Stalls and udders became dirtier as the neck rail became less restrictive (Fregonesi et al., 2009). These results were similar to a study by Tucker et al. (2005), and suggested that the decrease in stall and udder cleanliness most likely occurs because the neck rail ensures that when the cow stands up, she must take a step backwards, out of the stall. This restricts urination and defecation to the alley rather than to the stall surface. Decreased stall surface soiling results in increased udder cleanliness (Rendos et al., 1975; Zdanowicz et al., 2004).

Effects of Management on Lying Time

In addition to stall design, management factors can affect stall use. For example, lying time is decreased when cows are overstocked and there is insufficient lying space for the number of cows housed in the pen (Wierenga and Hopster, 1990; Fregonesi et al., 2007a).

Overstocking

Barns housing twice as many cows as there are free stalls should in theory allow all cows to achieve adequate lying times as cows typically only lie down for about 12 hours per day. However, dairy cows are herd animals and tend to synchronize their behaviour (DeVries and von Keyserlingk, 2005) including lying times (Fregonesi et al., 2007a). Fregonesi et al. (2007a) showed that as stocking density increased, or as the number of stalls per cow decreased, lying time also decreased. Cows engaged in more aggressive interactions and tried to displace each other from the free stalls (Fregonesi et al., 2007a). Feed is typically delivered to dairy cattle while they are being milked so that when they return to their pen after milking, they are motivated to remain standing (Fregonesi et al., 2007a). As Fregonesi et al. (2007a) described, this period of standing after milking is traditionally thought to improve udder health as it allows time for the teat ends to close, thereby minimizing bacteria that enter the teat canal and hopefully reducing mastitis. However, in Fregonesi et al.'s (2007a) study, latency to lie down also decreased; cows lie down upon returning from the milking parlour rather than eating feed. Therefore, when cows are overstocked at the free stalls, cows choose to forego eating fresh feed and instead claim a lying stall.

Quality of the Lying Surface

As described by Tucker et al. (2005), dairy cattle are descendants of animals that most likely make decisions about where to lie down based on the quality of the ground rather than making decisions based on "overhead spatial constraints", such as the neck rail. This idea suggests that dairy cattle behaviour may be particularly affected by the quality or type of the lying surface. Dairy cattle have also been shown to decrease lying time by 1.5 hours per day when there are insufficient amounts of bedding material on the stall surface (zero kg of sawdust versus 7.5 kg of sawdust on top of mattresses; Tucker and Weary, 2004) or when the stall surface is not maintained and becomes uneven (Drissler et al., 2005). Drissler et al. (2005) reported reductions in lying time of about 1.2 hours per day when sand bedding levels in free stalls were not maintained. Similarly, cows housed in tie stall barns also spend more time lying down as the depth of both straw and sawdust bedding increases (Tucker et al., 2009).

Lying time has also been affected by different types of bedding material and the cows' previous exposure to these different materials (Tucker et al., 2003). Tucker et al. (2003) found that cows lacking prior experience with sand bedding preferred to lie on sawdust while cows with experience on sawdust and sand did not show a distinct preference for either material. However, cows chose to lie on sawdust or sand over mattresses (Tucker et al., 2003). These results agree with other literature suggesting that cows prefer to lie on softer surfaces (Fulwider and Palmer, 2004).

Effects of Environment on Lying Time

In addition to the effects of management on lying time in dairy cows, changes in

lying behaviour are also dependent on environmental factors, such as ambient temperature (Keys et al., 1976; Shultz, 1984; Manninen et al., 2002; Cook et al., 2007). Intensively housed dairy cattle typically do not suffer from cold stress as the housing environment usually provides sufficient protection from the cold. Dairy cattle have a lower critical temperature ranging between -10 and -30°C, depending on their stage of lactation, and they can increase caloric intake to maintain body heat (Young, 1981).

In contrast, cows have more difficulty in coping with high temperatures, especially when combined with high humidity. The temperature humidity index (THI) can be used to combine these elements into a single index (Ravagnolo et al., 2000). Cows have been shown to spend less time lying down and become "heat stressed" when the THI is 72, corresponding to 25°C at 50% humidity (Ravagnolo et al., 2000). Temperatures above this level can have more detrimental effects on dairy cow welfare. Even short-term heat stress (THI > 72) has been shown to decrease milk production (Ominski et al., 2002).

Several studies have shown that dairy cattle spend more time standing in the alley and less time lying in the stall when environmental temperature increases (Keys et al., 1976; Shultz, 1984; Manninen et al., 2002; Cook et al., 2007). For example, Keys et al. (1976) reported reductions in lying time greater than seven hours per day when maximum temperatures were 15°C in the winter and 30°C in the summer. Igono et al. (1987) suggested that cows spend more time standing in the alley because this behaviour allows them to maximize evaporative cooling compared to when they are lying in stalls next to other heat-producing cows.

In addition to differences in temperature, different seasons result in changes in

day length. Longer photoperiods have been shown to increase milk production in lactating dairy cattle (Dahl et al., 1997; Miller et al., 2000). The effects of natural photoperiod on the lying behaviour of dairy cows have not been studied, but one study has suggested that artificial changes in photoperiod have no effect on lying behaviour (Tanida et al., 1984).

Lying Behaviour

While recording behaviours under different circumstances can help to identify how different stall elements or environmental factors affect stall usage, it is also important to consider factors such as how cows lie down are affected by stall design and the stall surface. Examining behaviour and observing cows in their natural settings can provide a better understanding of how intensive housing systems affect dairy cow behaviour.

Lying Down

Research has shown that stall design affects how a cow physically maneuvers from the standing to lying position (Ceballos et al., 2004). Ceballos et al. (2004) compared dairy cow behaviour when cows were housed in an open area versus a more restrictive lying area (the free stall cubicle). Using three dimensional kinematic analysis, this study illustrated that cows provided an open lying area made use of more lunge space than that typically provided in the free stall and thus cows were able to move more naturally.

In addition to how cows physically lie down, previous work has shown that dairy cows spend different amounts of time investigating the lying surface before actually

choosing to lie down (Krohn and Munksgaard, 1993; Tucker and Weary, 2004). For example, a study by Krohn and Munksgaard (1993) showed that cows spent five times longer examining the lying area when restricted to tie stalls compared to when cows were able to lie down on pasture. Tucker and Weary (2004) reported that cows spent more time head-swinging before lying down when the lying surface was covered with zero kg of sawdust compared to 7.5 kg of bedding. In this same study, cows spent 1.5 more hours per day lying down on the heavily bedded treatment, suggesting that more bedding was preferable to the cows and that head-swinging occurs for longer periods when the lying surface is undesirable (Tucker and Weary, 2004).

Lying Bouts

Previous work has reported that less suitable lying surfaces typically result in fewer lying bouts and less total lying time rather than shorter lying bouts (Tucker and Weary, 2004). Despite lying down more as described above, the study by Tucker and Weary (2004) showed that cows lie down less often (about 1.5 fewer lying bouts per day) in stalls with no bedding compared to stalls with 7.5 kg of bedding. However, the length of individual lying bouts remained constant across treatments.

Hernandez-Mendo et al. (2007) compared the lying times of cows housed in free stalls with those housed on pasture. Although cows restricted to pasture reduced their lying times by approximately one hour per day, they had three more bouts each day, compared to cows kept continuously in free stall barns. These results, combined with those of Tucker and Weary (2004), suggest that pasture may be a more suitable lying place and thus cows are more willing to transition between the standing and lying postures, despite the reduction in total lying time. Hernandez-Mendo et al. (2007) also

suggested that standing is less aversive to dairy cows on pasture and so cows reduce the overall amount of time they spend lying on pasture compared to when in a free stall barn; cows in a free stall barn may choose to lie down longer to avoid standing on hard floors. *Resting Behaviour*

Resting behaviour in cattle is defined as when the animal lies with its head flat on the side, or when the head is positioned back against its neck or on the ground (Krohn and Munksgaard 1993). While stall design and the stall surface affect how cows physically lie down, dairy cows have been shown to change their lying posture dependent on their overall housing environment rather than on the actual lying surface. Krohn and Munksgaard (1993) described the lying behaviour of dairy cows when kept on pasture, on deep-bedded loose housing, or in tie stalls with concrete or straw-covered lying surfaces. Cows spent the majority of time exhibiting "resting" postures when kept on pasture and the least amount of time displaying these behaviours when kept in tie stalls.

Interestingly, despite the overall decline in resting behaviour in tie stall housed cows compared to pasture, there was little change depending on the different lying surfaces provided in the different tie stall treatments. Presumably, the differences in lying posture arose because of tethering in the tie stall rather than the actual lying surface (Krohn and Munksgaard, 1993). Similarly, work by Bolinger et al. (1997) agrees with Krohn and Munksgaard (1993), reporting that cows changed their lying posture depending on whether or not they were restricted from lying down beforehand. Cows showed differences in posture as a result of lying deprivation and not as a consequence of differences in lying surface (Bolinger et al., 1997).

Lying Time and Bedding Dry Matter

Different types of bedding materials have different dry matter levels, and so a producer's choice of bedding and bedding management may result in a range of dry matter levels. Bedding dry matter appears to affect lying times in dairy cattle, but to date there has been limited work in this area.

Bedding becomes wet for a variety of reasons, including urination and defecation on the stall surface, manure tracked into the stall on the hooves of cattle, leaked milk, or even as a result of poorly maintained barns and leaky roofs. Keys et al. (1976) found that cows spent less time lying down on wetter bedding, but the different options were also of different bedding materials and it is impossible to ascertain whether cows chose to lie on one type of bedding because of its dry matter content or because of some other characteristic such as smell, insulating characteristics, or softness.

Fregonesi et al. (2007b) examined how dairy cows adjusted their behaviour when exposed to two different dry matter levels in sawdust bedding. In this study, cows spent five hours per day less lying in the stall on bedding containing 26.5% dry matter (wet bedding) compared to 86.4% dry matter (dry bedding). Thus cows are clearly able to distinguish between two extreme levels of bedding dry matter.

A recent survey of dairy farms in British Columbia (Ito, unpublished) showed that dairy cows are typically housed on a range of dry matter levels intermediate to the two options tested in the study by Fregonesi et al. (2007b). Thus, there is need for additional work that investigates intermediate levels of bedding dry matter more reflective of conditions observed on commercial dairy farms. Furthermore, while Fregonesi et al., (2007b) showed that cows decreased their lying time on wet bedding compared to dry

bedding, this study was conducted during the cool winter months; it is not known whether cows would also avoid wet bedding in warmer weather.

Objectives

The objectives of the current study were: 1) to determine the effects of various dry matter levels of sawdust bedding, 2) to assess the impact of season, and 3) to assess the interaction between bedding dry matter and season on the lying behaviour of Holstein dairy cows.

Hypothesis

It was hypothesized that dairy cows would spend less time lying down as dry matter of the sawdust bedding decreased and that cows would spend the most time lying on the driest bedding and the least time lying on the wettest bedding. It was also hypothesized that cows would respond more to wet bedding during the winter compared to the summer, due to the difference in ambient temperature.

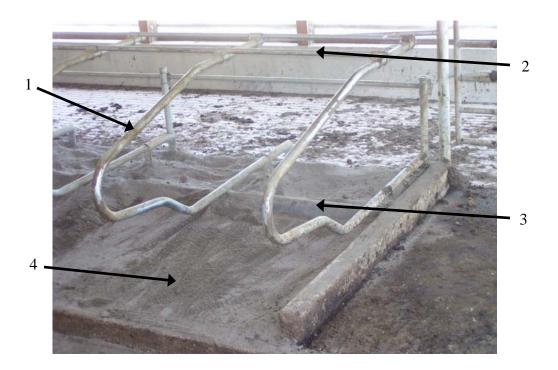


Figure 1.1. The elements typically found in a free stall include 1) stall partitions, 2) the neck rail, 3) the brisket board, and 4) the stall base, which is sometimes covered with bedding such as sand illustrated above.

References

- Bernardi, F., J. Fregonesi, C. Winckler, D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall-design paradox: neck rails increase lameness but improve udder and stall hygiene. J. Dairy Sci. 92:3074-3080.
- Bicalho, R. C., F. Vokey, H. N. Erb, and C. L. Guard. 2007. Visual locomotion scoring in the first seventy days in milk: impact on pregnancy and survival. J. Dairy Sci. 90:4586-4591.
- Bolinger, D. J., J. L. Albright, J. Morrow-Tesch, S. J. Kenyon, and M. D. Cunningham. 1997. The effects of restraint using self-locking stanchions on dairy cows in relation to behavior, feed intake, physiological parameters, health, and milk yield. J. Dairy Sci. 80:2411-2417.
- Booth, C. J., L. D. Warnick, Y. T. Grohn, D. O. Maizon, C. L. Guard, and D. Janssen. 2004. Effect of lameness on culling in dairy cows. J. Dairy Sci. 87:4115-4122.
- Ceballos, A., D. Sanderson, J. Rushen, and D. M. Weary. 2004. Improving stall design: Use of 3-D kinematics to measure space use by dairy cows when lying down. J. Dairy Sci. 87:2042-2050.
- Chapinal, N., A. M. de Passille, D. M. Weary, M. A. G. von Keyserlingk, and J. Rushen. 2009. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. J. Dairy Sci. 92:4365-4375.
- Cook, N. B., R. L. Mentink, T. B. Bennett, and K. Burgi. 2007. The effect of heat stress and lameness on time budgets of lactating dairy cows. J. Dairy Sci. 90:1674-1682.
- Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2004. Effect of free stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. J. Dairy Sci. 87:2912-2922.
- Cooper, M. D., D. R. Arney, and C. J. C. Phillips. 2007. Two- or four-hour lying deprivation on the behavior of lactating dairy cows. J. Dairy Sci. 90:1149-1158.
- Dahl, G. E., T. H. Elsasser, A. V. Capuco, R. A. Erdman, and R. R. Peters. 1997. Effects of long daily photoperiod on milk yield and circulating concentrations of insulinlike growth factor. J. Dairy Sci. 80:2784-2789.
- Dechamps, P., B. Nicks, B. Canart, M. Gielen, and L. Istasse. 1989. A note on resting behaviour of cows before and after calving in two different housing systems. App. Anim. Behav. Sci. 23:99-105.

DeVries, T. J. and M. A. G. von Keyserlingk. 2005. Time of feed delivery affects the

feeding and lying patterns of dairy cows. J. Dairy. Sci. 88:625-631.

- Drissler, M., M. Gaworski, C. B. Tucker, and D. M. Weary. 2005. Free stall maintenance: effects on lying behavior of dairy cattle. J. Dairy Sci. 88:2381-2387.
- Esslemont, R. J. and M. A. Kossaibati. 1996. Incidence of production diseases and other health problems in a group of dairy herds in England. Vet. Rec. 139:486-490.
- Food and Agriculture Organization of the United Nations (FAOStat). 2009. Food and agricultural commodities production. Retrieved August 9, 2009 from http://faostat.fao.org/site/339/default.aspx.
- Fraser, D. 2008. Understanding animal welfare: The science in its cultural context. Wiley-Blackwell, Oxford.
- Fraser, D., D. M. Weary, E. A. Pajor, and B. N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. Welf. 6:187-205.
- Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007a. Overstocking reduces lying time in dairy cows. J. Dairy Sci. 90:3349-3354.
- Fregonesi, J. A., D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2007b. Effects of bedding quality on lying behavior of dairy cows. J. Dairy Sci. 90:5468-5472.
- Fregonesi, J. A., M. A. G. von Keyserlingk, C. B. Tucker, D. M. Veira, and D. M. Weary. 2009. Neck-rail position in the free stall affects standing behavior and udder and stall cleanliness. J. Dairy Sci. 92:1979-1985.
- Fulwider, W. K. and R. W. Palmer. 2004. Use of impact testing to predict softness, cow preference, and hardening over time of stall bases. J. Dairy Sci. 87:3080-3088.
- Galindo, F. and D. M. Broom. 2000. The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. Res. in Vet. Sci. 69:75-79.
- Green, L. E., V. J. Hedges, Y. H. Schukken, R. W. Blowey, and A. J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. J. Dairy Sci. 85:2250-2256.
- Haley, D. B., J. Rushen, A. M. de Passille. 2000. Behavioural indicators of cow comfort: activity and resting behaviour of dairy cows in two types of housing. Can. J. Anim. Sci. 80:257-263.
- Hernandez-Mendo, O., M. A. G. von Keyserlingk, D. M. Veira, and D. M. Weary. 2007. Effects of pasture on lameness in dairy cows. J. Dairy Sci. 90:1209-1214.

- Hogan, J. S., V. L. Bogacz, L. M. Thompson, S. Romig, P. S. Schoenberger, W. P. Weiss, and K. L. Smith. 1999. Bacterial counts associated with sawdust and recycled manure bedding treated with commercial conditioners. J. Dairy Sci. 82:1690-1695.
- Igono, M. O., H. D. Johnson, B. J. Stevens, G. F. Krause, and M. D. Shanklin. 1987. Physiological, productive, and economic benefits of shade, spray, and fan system versus shade for Holstein cows during summer heat. J. Dairy Sci. 70:1069-1079.
- Jensen, M. B., L. J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. App. Anim. Behav. Sci. 90:207-217.
- Jensen, M. B., L. Munksgaard, L. J. Pedersen, J. Ladewig, and L. Matthews. 2004. Prior deprivation and reward duration affect the demand function for rest in dairy heifers. App. Anim. Behav. Sci. 88:1-11.
- Juarez, S. T., P. H. Robinson, E. J. DePeters, and E. O. Price. 2003. Impact of lameness on behaviour and productivity of lactating Holstein cows. App. Anim. Behav. Sci. 83:1-14.
- Keys, Jr., J. E., L. W. Smith, and B. T Weinland. 1976. Response of dairy cattle given a free choice of free stall location and three bedding materials. J. Dairy Sci. 59:1157-1162.
- Krohn, C. C., and L. Munksgaard. 1993. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments, II. Lying and lyingdown behaviour. App. Anim. Behav. Sci. 37:1-16.
- Manninen, E., A. M. de Passille, J. Rushen, M. Norring, and H. Salonieme. 2002. Preferences of dairy cows kept in unheated buildings for different kinds of cubicle flooring. App. Anim. Behav. Sci. 75:281-292.
- Metz, J. H. M. 1985. The reaction of cows to a short-term deprivation of lying. App. Anim. Behav. Sci. 13:301-307.
- Miller, A. R. E., R. A. Erdman, L. W. Douglass, and G. E. Dahl. 2000. Effects of photoperiodic manipulation during the dry period of dairy cows. J. Dairy Sci. 83:962-967.
- Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities - effects of time constraints on behaviour of dairy cows, *Bos taurus*. App. Anim. Behav. Sci. 92:3-14.

- Ominski, K. H., A. D. Kennedy, K. M. Wittenberg, and S. A. Mashtaghi Nia. 2002. Physiological and production responses to feeding schedule in lactating dairy cows exposed to short-term, moderate heat stress. J. Dairy Sci. 85:730-737.
- Ravagnolo, O., I. Misztal, and G. Hoogenboom. 2000. Genetic component of heat stress in dairy cattle, development of heat index function. J. Dairy Sci. 83:2120-2125.
- Rendos, J. J., R. J. Eberhart, and E. M. Kesler. 1975. Microbial populations on teat ends of dairy cows, and bedding materials. J. Dairy Sci. 58:1492-1500.
- Shultz, T. A. 1984. Weather and shade effects on cow corral activities. J. Dairy Sci. 67:868-873.
- Sogstad, A. M., O. Osteras, and T. Fjeldaas. 2006. Bovine claw and limb disorders related to reproductive performance and production diseases. J. Dairy Sci. 89:2519-2528.
- Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1996. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogen. 47:1179-1187.
- Statistics Canada. 2009. Cattle inventories, by province. Retrieved January 6, 2010 from http://www40.statcan.gc.ca/l01/cst01/prim50a-eng.htm.
- Tanida, H., L. V. Swanson, and W. D. Hohenboken. 1984. Effects of artificial photoperiod on eating behavior and other behavioral observations of dairy cows. J. Dairy Sci. 67:585-591.
- Tucker, C. B., and D. M. Weary. 2004. Bedding on geotextile mattresses: how much is needed to improve cow comfort? J. Dairy Sci. 87:2889-2895.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. J. Dairy Sci. 86:521-529.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2004. Free-stall dimensions: effects on preference and stall usage. J. Dairy Sci. 87:1208-1216.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2005. Influence of neck-rail placement on free-stall preference, use, and cleanliness. J. Dairy Sci. 88:2730-2737.
- Tucker, C. B., D. M. Weary, M. A. G. von Keyserlingk, and K. A. Beauchemin. 2009. Cow comfort in tie-stalls: increased depth of shavings or straw bedding increases lying time. J. Dairy Sci. 92:2684-2690.
- Tucker, C. B., G. Zdanowicz, and D. M. Weary. 2006. Brisket boards reduce free stall use. J. Dairy Sci. 89:2603-2607.

- US History Encyclopedia. 2010. Initiative. Retrieved March 25, 2010 from http://www.answers.com/topic/initiative.
- USDA. 2007. Dairy 2007, Part II: Changes in the U.S. dairy cattle industry, 1991-2007. No. N481.0308. USDA-Anim. Plant Health Inspection Serv.-Vet. Serv., Centers for Epidemiol. Anim. Health, Fort Collins, CO.
- Walker, S. L., R. F. Smith, J. E. Routly, D. N. Jones, M. J. Morris, and H. Dobson. 2008. Lameness, activity time-budgets, and estrus expression in dairy cattle. J. Dairy Sci. 91:4552-4559.
- Webster, J. 1986. Health and welfare of animals in modern husbandry systems dairy cattle. In Prac. May 85-89.
- Wierenga, H. K. and H. Hopster. 1990. The significance of cubicles for the behaviour of dairy cows. App. Anim. Behav. Sci. 26:309-337.
- Young, B. A. 1981. Cold stress as it affects animal production. J. Anim. Sci. 52:154-163.
- Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M. A. G. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J. Dairy Sci. 87:1694-1701.
- Zehner, M. M., R. J. Farnsworth, R. D. Appleman, K. Larntz, and J. A. Springer. 1986. Growth of environmental mastitis pathogens in various bedding materials. J. Dairy Sci. 69:1932-1941.

CHAPTER 2: Effects of Sawdust Bedding Dry Matter on Lying Behaviour of Dairy Cows: A Dose Dependent Response² Introduction

The lying surface provided for dairy cows can affect lying behaviour. The type (Keys et al., 1976; Tucker et al., 2003), amount (Tucker and Weary, 2004; Drissler et al., 2005), and dryness (% DM; Fregonesi et al., 2007b) of bedding can affect how much time cows spend lying down.

Bedding becomes wet when cows urinate and defecate, leak milk, and enter the lying area with dirty, wet hooves. Rain can enter the barn from the sides or through the open doors, and feed-line soakers can add moisture to bedding. Wet bedding can reduce the amount of time cows spend lying in free stalls. Fregonesi et al. (2007b) found that dairy cows show a strong preference for dry bedding; cows spent five hour per day less time lying down when they only had access to stalls with wet bedding compared to when they had access to stalls with dry bedding. In the same study, cows spent more time standing outside of the stall when restricted to wet bedding compared to when they had access to dry bedding. Yet, Fregonesi et al. (2007b) compared two extremes of moisture content (86.4 vs. 26.5% dry matter) and it is unknown how cows respond to intermediate moisture levels more typically found on commercial farms.

Several studies have examined the effect of season on lying behaviour, and for the most part, these studies focused on the obvious difference in temperature between seasons. Lactating dairy cows spend less time lying down when the temperature is high

² A version of this chapter has been published. Reich, L. J., D. M. Weary, D. M. Veira, and M. A. G. von Keyserlingk. 2010. Effects of sawdust bedding dry matter of lying behavior of dairy cows: A dose-dependent response. J. Dairy Sci. 93:1561-1565.

(Keys et al., 1976; Shultz, 1984; Cook et al., 2007), perhaps because standing improves their ability to use evaporative cooling. Cows might use conductive cooling by lying down on wet surfaces, but to our knowledge this idea has not been tested. There may be other differences between seasons that cause changes in behaviour. For example, photoperiod affected milk production (Dahl et al., 1997) and may affect lying behaviour as well.

The objectives were to determine 1) the effects of a range of moisture levels in sawdust bedding, and 2) the interaction between season and bedding moisture on the lying behaviour of Holstein dairy cows.

Materials and Methods

Animals, Housing and Diet

The study was conducted at the University of British Columbia's Dairy Education and Research Centre in Agassiz, British Columbia, Canada during August and September 2008 (summer) and replicated in January and February 2009 (winter). For each of the two replicates, 15 pregnant, non-lactating Holstein dairy cows were randomly assigned to five groups of three animals. Cows entered the experiment at the beginning of a target 60 day dry period. For the summer replicate, groups were balanced for mean (\pm SD) parity (2.2 \pm 1.1), days to expected date of calving (56.1 \pm 9.3 days), body weight (723 \pm 67 kg), and body condition score (3.3 \pm 0.4; scored from one to five following Edmonson et al., 1989). For the winter replicate, groups were balanced for mean parity (2.3 \pm 1.4), days to expected date of calving (59.1 \pm 15.7 days), body weight (727 \pm 116 kg), and body condition score (3.3 \pm 0.3).

Lameness affected standing behaviour, especially for cows housed on mattresses (Cook et al., 2004), so cows were only included in the experiment if they were not clinically lame (i.e., gait score < 3; Flower and Weary, 2006). Any cow that became lame over the course of the experiment was removed. Cows were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

The replicates were carried out in a naturally ventilated wooden frame barn (width = 38 m, length = 156 m) with a north-south orientation and curtained sidewalls. Each experimental pen measured 9.5 m wide by 12.3 m long with 12 stalls configured in 2 rows tail-to-tail. Nine stalls were blocked with chains to prevent cows from entering them and so that each pen had three stalls available for the three cows. The bed of each stall was 2.6 m in length. Stalls were separated (1.2 m center-to-center) with Y2K free stall partitions (Artex, Langley, British Columbia, Canada). The neck rail was positioned 1.2 m above the stall surface, and 1.5 m from the rear curb of the stall. The brisket board (Poly Pillow, Promat Ltd., Seaforth, Ontario, Canada) was positioned 1.7 m from the rear curb of the stall. The stall was covered with a geotextile mattress and bedded with 0.1 m kiln-dried sawdust (approximately 7.5 kg/stall). The rest of the flooring in the pen was covered with textured rubber. The alleys were 3.5 m wide at the feed bunk and 3.0 m wide between the two rows of stalls. Alleys were scraped six times per day with an automatic scraper.

Each pen had headlocks positioned 60 cm center-to-center at the feed bunk with a total of 9.5 m of bunk space per pen. Cows were given ad libitum access to a total mixed ration (TMR) consisting of 44.4% grass silage, 19.1% corn silage, 27.8% ryegrass seed straw, and 8.7% concentrate mix on a dry matter basis. The composition of the TMR was

46.6% dry matter and contained (on a dry matter basis) 13.4% CP, 35.9% ADF, and 55.6% NDF. Fresh feed was provided once daily at 0800 hours and feed was pushed up twice daily. A self-filling trough was used to provide water ad libitum.

Treatments and Experimental Design

Five moisture levels were tested for both replicates: four were prepared by mixing 1.7, 4.0, 9.0, and 15.5 L of water with 7.5 kg of sawdust in a cement mixer and the fifth consisted of kiln-dried sawdust without any added water. Bedding was replaced twice daily at 0800 and 1800 hours, and samples were collected before and after the bedding treatments were placed in the stalls. Samples were taken from each stall, pooled within treatments, and frozen in plastic bags until they were oven-dried at 55° C for two days. Bedding samples from the five treatments were (\pm SD) 34.7 \pm 3.8, 43.9 \pm 4.0, 62.2 \pm 6.3, 74.2 \pm 6.4, and 89.8 \pm 3.7 % dry matter. To correspond with the earlier literature on bedding moisture, bedding treatments will hereafter be referred to in bedding dry matter.

Groups were acclimatized to the test pens for five days on kiln-dried sawdust and were then exposed to each of the five treatments using a five by five Latin square design. Groups remained in the same pen throughout the course of the replicates and treatments changed within each pen. Each group was tested on each treatment for four days, with one day between each treatment on kiln-dried sawdust.

Behaviour

Behaviour was recorded 24 hours per day using two cameras (Panasonic WV-BP334 24V) placed above the feed bunk and above the stalls for each pen. The cameras were attached to a video multiplexer (Panasonic WJ FS616C) and time-lapse recorder (Panasonic AG 6540; Panasonic, Mississauga, Ontario, Canada). Red lights (100 W)

were hung adjacent to the cameras to facilitate video recording at night. Cows were marked with unique symbols using hair dye to identify individuals within each group if necessary. Video recordings were scanned at five minute intervals; each scan recorded if the cow was lying in the stall, lying in the alley, perching (standing with two hooves in the stall), standing with four hooves in the stall, standing elsewhere in the pen, or feeding (when the cow's head was in the headlock and ears were past the feed barrier). Behaviour was recorded for two of the four days during each treatment period. During the summer replicate, behaviour was recorded on day one and four of each treatment period. Due to difficulties with recording during the winter replicate, behaviour was recorded for two days chosen at random for each experimental period. Results were compared between different days of the experimental period for each season, and days within each experimental period did not have an effect on behaviour.

Temperature

A data logger (HOBO Temperature/Relative Humidity Data Logger, Onset Computer Corporation, Pocasset, MA) that recorded temperatures every three minutes was placed in the center of the barn to record daily minimum and maximum temperatures.

Statistical Analysis

The UNIVARIATE procedure (version 9.1, SAS Institute, 2003) was used to screen the data for outliers and normality. One cow, treated for lameness and identified as an extreme outlier for time spent standing fully in the stall, was removed from the data set. The remaining data were averaged by group (the experimental unit) and treatment, such that each group contributed one observation for each treatment level in the statistical

analysis.

The MIXED procedure (version 9.1, SAS Institute, 2003) was used to test the fixed effect of season (one degree of freedom), dry matter treatment (continuous, one degree of freedom) and the season by dry matter interaction (one degree of freedom), with group specified as a random effect. The interaction between season and dry matter treatment was never significant and will not be discussed further.

The REG procedure (version 9.1, SAS Institute, 2003) was used to calculate the coefficients of determination between lying time and temperature and day length, separately for each season. Temperature and day length are two obvious sources of variation within and between seasons.

Results

Bedding Dry Matter

Cows spent more time lying in the stall when sawdust bedding was drier (Figure 2.1A; P = 0.011). Cows spent 10.4 ± 0.4 hours per day lying in the stall on the wettest bedding (34.7% dry matter) versus 11.5 ± 0.4 hours per day on the driest treatment (89.8% dry matter). When bedding was wet, cows appeared to compensate for reduced lying time by spending more time standing idle in the alley (not including feeding time). With drier treatments time spent standing in the alley decreased by about 13% (Figure 2.1B; P = 0.015) because cows spent more time lying in the stalls. No other behaviours varied with the bedding dry matter. Cows averaged 1.5 ± 0.1 hour per day perching in the stall, 0.2 ± 0.1 hour per day standing in the stall, and 5.5 ± 0.1 hours per day feeding (Appendix 1, Table A1.1 and Appendix 2, Table A2.1).

During the summer replicate, three of the cows occassionally lay down in the alley, but none of the cows in the winter replicate ever exhibited this behaviour. Because only a few cows showed this behaviour and it occurred on a variety of treatments during different treatment periods, lying in the alley was attributed to differences among cows, rather than bedding dry matter or season, and will not be discussed further.

Season

The average (\pm SD) minimum and maximum temperatures were 2.6 \pm 2.0° C and 6.8 \pm 2.2° C, respectively, for the winter replicate and 13.3 \pm 2.5° C and 22.6 \pm 4.1° C, respectively, for the summer replicate. There were seasonal effects on lying in the stall and standing in the alley. The time cows spent lying in stalls was 2.2 hours greater in the winter than in the summer (P = 0.0003; Figure 2.1A), and cows spent 1.4 hours less time standing outside of the stall in the winter months (P = 0.0146; Figure 2.1B). There were no seasonal effects on perching or standing in the stall, but cows averaged 5.0 \pm 0.2 hours per day feeding in the winter versus 5.8 \pm 0.2 hours per day in the summer months (P < 0.0001; Figure 2.1C).

There was no effect of temperature on lying time within either winter or summer and no effect of day length on lying time during the winter. As day length increased during the summer, cows tended to spend less time lying down ($R^2 = 0.12$, P < 0.09; Appendix 3, Figures A3.1-4).

Discussion

Bedding Dry Matter

The results confirmed that wet sawdust bedding reduces the amount of time cows

spend lying down in the stall, and supports previous studies (Keys et al., 1976, Fregonesi et al., 2007b). Cows spent 1.1 hours per day less time lying on the wettest treatment compared to the driest. These results support other studies examining stall design and cow comfort.

Changes in stall design and management typically increased or reduced lying times by zero to three hours per day; examples include changes in bedding materials (Tucker et al., 2003), stall width and length (Tucker et al., 2004), amount of bedding material (Tucker and Weary, 2004), neck rail placement (Tucker et al., 2005), stall surface and depth of bedding material (Drissler et al., 2005), brisket board (Tucker et al., 2006), and stocking density (Fregonesi et al., 2007a). Yet, in a study comparing moisture levels, Fregonesi et al. (2007b) reported a decrease of five hours per day in lying time on wet bedding (26.5% dry matter) compared to dry bedding (86.4% dry matter). The smaller difference between treatments in the current study may be partially due to differences in the moisture levels used. The lowest dry matter ($34.7 \pm 3.8\%$ dry matter) was still about 8% drier than the 'wet' treatment (26.5% dry matter) in Fregonesi et al. (2007b).

Bedding materials containing low percentages of dry matter (29% dry matter in dewatered manure solids; Keys et al., 1976, and 26.5% dry matter in sawdust bedding; Fregonesi et al., 2007b) reduced lying times relative to bedding materials containing high percentages of dry matter (81% dry matter in sawdust and 90% dry matter in dehydrated manure solids; Keys et al., 1976, and 86.4% dry matter in sawdust; Fregonesi et al., 2007b). The current study examined the effects of intermediate levels of dry matter in sawdust bedding and showed a decrease in the amount of time cows spend lying in the

stall as the dry matter of sawdust decreases. Yet, this decrease in lying time was modest until dry matter dropped below approximately 60% dry matter, suggesting that cows may not have a strong preference for drier bedding when all options are relatively dry (i.e., between 60% dry matter and 90% dry matter).

Effect of Season

Lying time was lower in the summer than the winter. Dairy cattle are known to spend less time lying down when heat stressed (Shultz, 1984), or mildly heat stressed (i.e., THI > 68; Cook et al., 2007). There was no relationship between temperature and lying time within either season, suggesting that some other seasonal factor was involved. There was no relationship between lying time and day length during the winter, but lying times tended to decrease as day length increased during the summer replicate. Thus when dairy cows are not heat stressed, day length may be a better predictor of lying time than temperature.

Dry matter intake is known to decrease as a result of heat stress (West, 2003). Dry matter intake was not measured, but the time spent feeding was lower in the winter than summer. If cows had been heat stressed during the summer, the opposite result would have been expected (i.e., decreased feeding time during the summer).

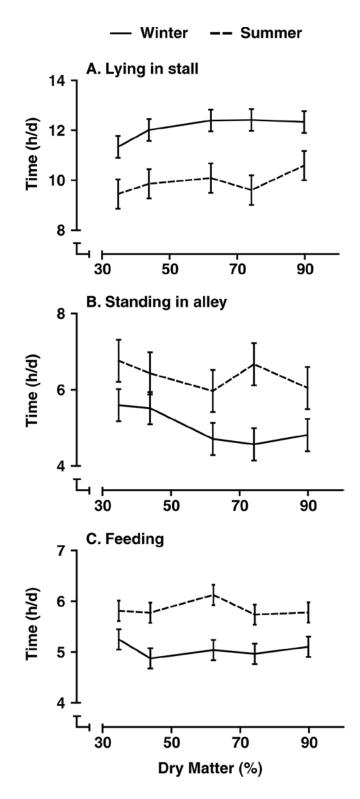


Figure 2.1. Time (hours/day) spent A) lying in the stall, B) standing in the alley, and C) feeding, shown separately for summer and winter replicates. Data were averaged from five groups, for two days on each treatment using a Latin-square design.

References

- Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol. 1. E. D. Olfert, B. M. Cross, and A. A. McWilliam, ed. CCAC, Ottawa, Ontario, Canada.
- Cook, N. B., R. L. Mentink, T. B. Bennett, and K. Burgi. 2007. The effect of heat stress and lameness on time budgets of lactating dairy cows. J. Dairy Sci. 90:1674-1682.
- Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2004. Effect of free stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. J. Dairy Sci. 87:2912-2922.
- Dahl, G. E., T. H. Elsasser, A. V. Capuco, R. A. Erdman, and R. R. Peters. 1997. Effects of a long daily photoperiod on milk yield and circulating concentrations of insulin-like growth-factor 1. J. Dairy Sci. 80:2784-2789.
- Drissler, M., M. Gaworski, C. B. Tucker, and D. M. Weary. 2005. Free stall maintenance: effects on lying behavior of dairy cattle. J. Dairy Sci. 88:2381-2387.
- Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.
- Flower, F. C. and D. M. Weary. 2006. Hoof pathologies influence kinematic measures of dairy cow gait. J. Dairy Sci. 89:139-146.
- Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007a. Overstocking reduces lying time in dairy cows. J. Dairy Sci. 90:3349-3354.
- Fregonesi, J. A., D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2007b. Effects of bedding quality on lying behavior of dairy cows. J. Dairy Sci. 90:5468-5472.
- Keys, J. E., L. W. Smith, and B. T. Weinland. 1976. Response of dairy cattle given a free choice of free stall location and three bedding materials. J. Dairy Sci. 59:1157-1162.
- SAS Institute Inc. 2003. SAS Users Guide. Version 9.1. SAS Institute Inc., Cary, NC.
- Shultz, T. A. 1984. Weather and shade effects on cow corral activities. J. Dairy Sci. 67:868-873.
- Tucker, C. B., and D. M. Weary. 2004. Bedding on geotextile mattresses: How much is needed to improve cow comfort? J. Dairy Sci. 87:2889-2895.

- Tucker, C. B., D. M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. J. Dairy Sci. 86:521-529.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2004. Free-stall dimensions: Effects on preference and stall usage. J. Dairy Sci. 87:1208-1216.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2005. Influence of neck-rail placement on free-stall preference, use, and cleanliness. J. Dairy Sci. 88:2730-2737.
- Tucker, C. B., G. Zdanowicz, and D. M. Weary. 2006. Brisket boards reduce free stall use. J. Dairy Sci. 89:2603-2607.
- West, J. W. 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86:2131-2144.

CHAPTER 3: Conclusions

Contributions

Stall design (Tucker et al., 2004; Tucker et al., 2006) and especially stall surface (Tucker et al., 2003; Tucker and Weary, 2004) are important factors affecting the amount of time cows spend lying down. Furthermore, lying time is an important behaviour that cattle are motivated to perform (Jensen et al., 2004; Jensen et al., 2005). One aspect of the lying surface that affects lying time is bedding moisture (Keys et al., 1976; Fregonesi et al., 2007). Moisture levels in bedding can increase when cows urinate or defecate in the stall, when cows leak milk, or because of leaky barns.

According to results of a survey of British Columbia dairy farms in 2008 (Ito, unpublished), moisture contents of bedding ranged between the more extreme dry matter levels tested in the studies by Fregonesi et al. (2007) and Keys et al. (1976). Fregonesi et al. (2007) compared two extreme dry matter levels and demonstrated that cows reduced their lying time by about five hours per day on very wet bedding (26.5% dry matter) compared to dry bedding. Similarly, a study by Keys et al. (1976) compared two different dry matter levels in bedding materials, but examined two types of manure solids (dewatered manure solids at 29% dry matter and dehydrated manure solids at 90% dry matter). While cows spent less time lying on wet bedding compared to dry bedding, it was not clear if cows changed their lying time in response to bedding dry matter or because the dewatered manure solids had a more noticeable odor than the dehydrated manure which may have discouraged cows from choosing to lie down (Keys et al., 1976). Although these two studies have provided some insights into the effects of bedding dry matter on lying behaviour, the moisture levels examined were extreme (26.5% dry matter

versus 86.4% dry matter in Fregonesi et al., 2007; 29% dry matter versus 90% dry matter in Keys et al., 1976).

The purpose of this thesis was to examine a range of dry matter levels in sawdust, a commonly used bedding material, between the two extremes that have been previously tested. My study showed that cows decrease the amount of time they spend lying down when bedding dry matter decreases, but this change is relatively moderate when bedding is above about 60% dry matter.

Another aim of my study was to compare the effects of a range of dry matter levels on the lying time of dairy cows across summer and winter seasons. This was in response to gaps in the previous literature. Previous work on the effects of season have reported that cows typically spend less time lying down during the summer compared to the winter (Keys et al., 1976; Cook et al., 2007), perhaps because standing up allows cows to cool off by increasing evaporative cooling (Igono et al., 1987). While the cows observed in my study spent less time lying down overall during the summer compared to the winter, the effect of bedding moisture did not vary with season.

If the effect of season was due to differences in ambient temperatures, we might also expect that cows' lying behaviour would vary in response to changes in ambient temperature even within the summer season. However, I found no effect of temperature on lying behaviour within the summer, suggesting that other factors may be at play; one potential factor is day length. Previous work has shown that day length can affect dairy cattle reproduction, lactation, and immunology (Collier et al., 2006), but the results of this thesis suggest that day length does not have a major effect on lying time.

Future Research

Future research should focus on the longer term health implications of reduced lying time as a result of poor housing conditions. The cows in this study reduced their lying time by about one hour in response to the wettest treatments, but it is not clear what the longer term effects of reduced lying time would be on the animals' welfare. As reviewed above, previous work has shown that cows compensate for lost lying time (Munksgaard et al., 2005) and spend more time standing when deprived of lying down for approximately two hours (Cooper et al., 2007). Increased standing outside of stalls also occurs when cows are provided with an undesirable stall surface (Tucker et al., 2006; Fregonesi et al., 2007). Additionally, Colam-Ainsworth et al. (1989) compared two identical herds of dairy heifers and reported a higher incidence of lameness in the herd housed in free stalls with less bedding. The authors attributed increased lameness to increased standing times, most likely as a result of insufficient bedding (Colam-Ainsworth et al., 1989).

If the stall surface is wet, cows may also be less motivated to lie down to alleviate the pain associated with lameness. Lame cows have been shown to lie down more often (Juarez et al., 2003; Chapinal et al., 2009), presumably to avoid standing on hard alley surfaces. Therefore, examining the longer term effect of small reductions in lying time, such as one hour per day, on the development of lameness could provide producers with more motivation to change bedding more often. I strongly encourage future research in this area.

Another long term effect to examine is how wet bedding affects mastitis prevalence. According to a study by Zehner et al. (1986), the cleanliness and dryness of

bedding are important factors in controlling bacterial growth, and bedding moisture and the amount of nutrients have been shown to impact the amount of bacteria on the stall surface (Zdanowicz et al., 2004). If damp or wet bedding is associated with higher levels of bacterial growth, we can assume that cows kept on damp or wet bedding would be at higher risk of becoming infected with mastitis-causing bacteria.

Some of the reasons bedding becomes wet include leaky barns or rain entering the barn through open doors. Cows also track manure into stalls and urinate and defecate on the stall surface. However, to maintain consistent dry matter levels throughout my experiment, treatments were applied using fresh sawdust and clean water and bedding was changed twice daily. In the study by Keys et al. (1976) the authors suggested that cows may have chosen to lie down less on the wettest bedding material because of possible odor. An interesting experiment would be to see how cows would respond to declining bedding quality if sawdust bedding was not changed as often as it was in my trial, but rather bedding dry matter declined as a result of natural soiling. Perhaps cows would be more sensitive to wet bedding if it was wet as a result of soiling and also had a stronger odor.

Previous work has shown that poor quality lying surfaces result in a decreased number of lying bouts (Tucker and Weary, 2004). While I recorded behaviour using time lapse video, which provides an excellent estimate of daily lying time, lying bouts could not be recorded using this form of data collection. Therefore future research should investigate the effects of wet bedding on the number of lying bouts.

The placement of the video cameras did not allow for the observation of dairy cow behaviour as they approach the stall (e.g. head swinging behaviour). However, I

would predict that cows spend more time head-swinging and more time investigating the stall surface before lying down; cows have been shown to spend more time investigating the stall surface and more time head-swinging before lying down on less preferable lying surfaces (Krohn and Munksgaard, 1993; Tucker and Weary, 2004). These may be useful indicators to include in future analysis of how bedding quality affects lying behaviour.

More research is needed to determine exactly how seasonal factors affect lying behaviour when dairy cattle are kept in moderate climates and are therefore not heat stressed. Season had an impact on the amount of time cows spent lying down; however it was unclear if this behavioural change was a result of temperature. Dairy cows have been reported to shift their behaviour as a result of mild heat stress at a THI of 68 or around 21°C (Cook et al., 2007). While maximum daily temperatures recorded during the summer experiment reached above 21°C, THI was never above 67, suggesting that the cows in my experiment were not mildly heat stressed. Additionally, linear regression analysis showed no relationship between temperature or day length and lying time within either season, but there was an obvious difference between the two seasons. Both day length and temperature varied with season, and this study covered only a narrow range of temperature and day length within season.

Controlled experiments may be necessary to study each seasonal variable individually and determine which is responsible for altering lying behaviour when cows are not heat stressed. To answer this question, temperature could be variable within season and day length could be controlled with artificial photoperiods in an enclosed barn. Similarly, temperature could be held constant and artificial lighting could be used to simulate changes in day length.

Summary

The results of this thesis support the recommendation that dairy producers be encouraged to change sawdust bedding when it falls below 60% dry matter. Previous stall surface assessments have focused on the type and amount of materials used (Nordlund and Cook, 2003), but little focus has been placed on evaluating the dry matter of the lying surface. Therefore, producers must be made aware that wet bedding has the potential to decrease lying time, similarly to restrictive hardware elements and bedding material type. Different types of bedding materials inherently have different dry matter levels (e.g. dehydrated manure and dewatered manure; Keys et al., 1976), so consideration of bedding dry matter, even when bedding is freshly placed in the stall, is an important factor in good stall management.

Research over the last few years has also examined the effects of moving neck rails to less restrictive positions to allow cows to stand in the stalls rather than in the alleys (Tucker et al., 2005; Bernardi et al., 2009; Fregonesi et al., 2009). Several studies have also reported improved locomotion scores with less restrictive neck rails (Bernardi et al., 2009). While these studies did not report changes in lying time, they did report increased soiling of the stall surface (Tucker et al., 2005; Bernardi et al., 2009; Fregonesi et al., 2009). As these experiments were carried out on a research farm, the environment was highly controlled and stalls were most likely maintained to a greater extent than most commercial dairy farms. Therefore, producers should be cautioned that while moving the neck rails to less restrictive positions may reduce lameness, increased stall maintenance is necessary to keep bedding dry and maintain lying times. Overall, the results from this master's thesis suggest that minimizing wet lying surfaces will likely increase lying times.

References

- Bernardi, F., J. Fregonesi, C. Winckler, D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall-design paradox: neck rails increase lameness but improve udder and stall hygiene. J. Dairy Sci. 92:3074-3080.
- Chapinal, N., A. M. de Passille, D. M. Weary, M. A. G. von Keyserlingk, and J. Rushen. 2009. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. J. Dairy Sci. 92:4365-4375.
- Colam-Ainsworth, P., G. A. Lunn, R. C. Thomas, and R. G. Eddy. 1989. Behaviour of cows in cubicles and its possible relationship with laminitis in replacement dairy heifers. Vet. Rec. 125:573-575.
- Collier, R. J., G. E. Dahl., and M. J. VanBaale. 2006. Major advances associated with environmental effects on dairy cattle. J. Dairy Sci. 89:1244-1253.
- Cook, N. B., R. L. Mentink, T. B. Bennett, and K. Burgi. 2007. The effect of heat stress and lameness on time budgets of lactating dairy cows. J. Dairy Sci. 90:1674-1682.
- Cooper, M. D., D. R. Arney, and C. J. C. Phillips. 2007. Two- or four-hour lying deprivation on the behavior of lactating dairy cows. J. Dairy Sci. 90:1149-1158.
- Fregonesi, J. A., D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2007. Effects of bedding quality on lying behavior of dairy cows. J. Dairy Sci. 90:5468-5472.
- Fregonesi, J. A., M. A. G. von Keyserlingk, C. B. Tucker, D. M. Veira, and D. M. Weary. 2009. Neck-rail position in the free stall affects standing behavior and udder and stall cleanliness. J. Dairy Sci. 92:1979-1985.
- Igono, M. O., H. D. Johnson, B. J. Steevens, G. F. Krause, and M. D. Shanklin. 1987. Physiological, productive, and economic benefits of shade, spray, and fan system versus shade for Holstein cows during summer heat. J. Dairy Sci. 70:1069-1079.
- Jensen, M. B., L. J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. App. Anim. Behav. Sci. 90:207-217.
- Jensen, M. B., L. Munksgaard, L. J. Pedersen, J. Ladewig, and L. Matthews. 2004. Prior deprivation and reward duration affect the demand function for rest in dairy heifers. App. Anim. Behav. Sci. 88:1-11.

- Juarez, S. T., P. H. Robinson, E. J. DePeters, and E. O. Price. 2003. Impact of lameness on behaviour and productivity of lactating Holstein cows. App. Anim. Behav. Sci. 83:1-14.
- Keys, Jr., J. E., L. W. Smith, and B. T Weinland. 1976. Response of dairy cattle given a free choice of free stall location and three bedding materials. J. Dairy Sci. 59:1157-1162.
- Krohn, C. C., and L. Munksgaard. 1993. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments, II. Lying and lyingdown behaviour. App. Anim. Behav. Sci. 37:1-16.
- Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities - effects of time constraints on behaviour of dairy cows, *Bos taurus*. App. Anim. Behav. Sci. 92:3-14.
- Nordlund, K. and N. B. Cook. 2003. A flowchart for evaluating dairy cow free stalls. Bov. Prac. 37:89-96.
- Tucker, C. B., and D. M. Weary. 2004. Bedding on geotextile mattresses: How much is needed to improve cow comfort? J. Dairy Sci. 87:2889-2895.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. J. Dairy Sci. 86:521-529.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2004. Free-stall dimensions: effects on preference and stall usage. J. Dairy Sci. 87:1208-1216.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2005. Influence of neck-rail placement on free-stall preference, use, and cleanliness. J. Dairy Sci. 88:2730-2737.
- Tucker, C. B., G. Zdanowicz, and D. M. Weary. 2006. Brisket boards reduce free stall use. J. Dairy Sci. 89:2603-2607.
- Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M. A. G. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J. Dairy Sci. 87:1694-1701.
- Zehner, M. M., R. J. Farnsworth, R. D. Appleman, K. Larntz, and J. A. Springer. 1986. Growth of environmental mastitis pathogens in various bedding materials. J. Dairy Sci. 69:1932-1941.

APPENDIX 1: Behavioural Measures

Table A1.1. This table shows the hours per day (mean \pm SE) cows spent performing each
behaviour on each bedding dry matter level, averaged across seasons.

		Beł	naviours (Mean ±	SE)	
Bedding Dry Matter (%)	Lying in Stall (h/d)	Standing in Alley (h/d)	Perching in Stall (h/d)	Standing in Stall (h/d)	Feeding (h/d)
34.7	10.4 ± 0.4	6.2 ± 0.3	1.4 ± 0.1	0.2 ± 0.1	5.6 ± 0.1
43.9	10.9 ± 0.4	6.0 ± 0.3	1.4 ± 0.1	0.2 ± 0.1	5.3 ± 0.1
62.2	11.2 ± 0.4	5.3 ± 0.3	1.4 ± 0.1	0.3 ± 0.1	5.6 ± 0.1
74.2	11.0 ± 0.4	5.6 ± 0.3	1.6 ± 0.1	0.2 ± 0.1	5.4 ± 0.1
89.8	11.5 ± 0.4	5.4 ± 0.3	1.4 ± 0.1	0.2 ± 0.1	5.4 ± 0.1

APPENDIX 2: Behavioural Measures by Season

Table A2.1. This table shows the hours per day (mean \pm SE) cows spent performing each behaviour on each bedding dry matter level,

by season.

				Behavio	ours by season	$(Mean \pm SE)$)			
Bedding Dry Matter (%)		in Stall /d)	C C	; in Alley /d)	Perching (h/			g in Stall /d)		ding /d)
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
34.7	9.5 ± 0.6	11.3 ± 0.4	6.8 ± 0.6	5.6 ± 0.4	1.3 ± 0.2	1.6 ± 0.2	0.2 ± 0.1	$0.3 \pm < 0.1$	5.9 ± 0.2	5.3 ± 0.2
43.9	9.9 ± 0.6	12.0 ± 0.4	6.4 ± 0.6	5.5 ± 0.4	1.5 ± 0.2	1.4 ± 0.2	0.2 ± 0.1	$0.2 \pm < 0.1$	5.8 ± 0.2	4.9 ± 0.2
62.2	10.1 ± 0.6	12.4 ± 0.4	6.0 ± 0.6	4.7 ± 0.4	1.3 ± 0.2	1.6 ± 0.2	0.3 ± 0.1	$0.3 \pm < 0.1$	6.1 ± 0.2	5.0 ± 0.2
74.2	9.6 ± 0.6	12.4 ± 0.4	6.7 ± 0.6	4.6 ± 0.4	1.4 ± 0.2	1.8 ± 0.2	0.2 ± 0.1	$0.2 \pm < 0.1$	5.7 ± 0.2	5.0 ± 0.2
89.8	10.6 ± 0.6	12.3 ± 0.4	6.1 ± 0.6	4.8 ± 0.4	1.3 ± 0.2	1.5 ± 0.2	0.2 ± 0.1	$0.2 \pm < 0.1$	5.8 ± 0.2	5.1 ± 0.2

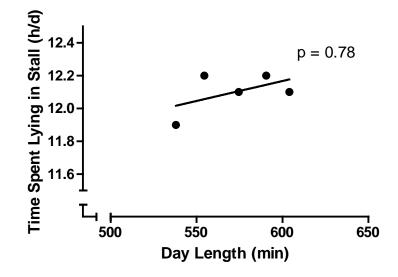


Figure A3.1. Day length did not have a significant relationship with the amount of time cows spent lying in the stall during the winter.

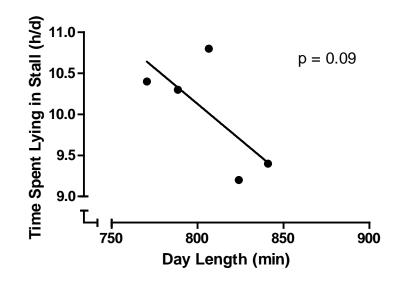


Figure A3.2. Cows tended to decrease their lying time as days became longer during the summer.

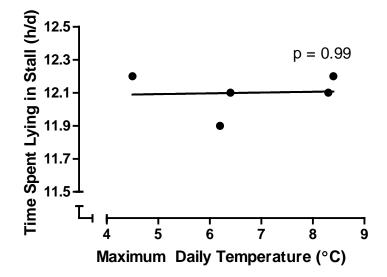


Figure A3.3. Maximum temperatures for each treatment period were not significantly related to the amount of time cows spent lying in the stall during the winter.

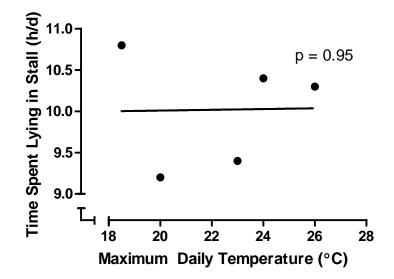


Figure A3.4. Maximum temperatures for each treatment period did not have a significant relationship with the amount of time cows spent lying in the stall during the summer.

APPENDIX 4: Animal Care Certificate



THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Nu	mber: A05-1906
Investigator or	Course Director: Marina Andrea G. von Keyserlingk
Department: La	and and Food Systems
Animals:	
	rs Cattle 96 rs dairy 225
Start Date: F	ebruary 1, 2006 Approval Date: May 14, 2009
Funding Source	es:
Funding Agency:	Unifeed Ltd.
Funding Title:	Evaluation and Validation of Cow Comfort on B.C. Farms
Funding Agency:	Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title:	NSERC/SPCA-BCVMA-Animal Industries Junior Industrial Research Chair in Animal Welfare (research)
Funding Agency:	Investment Agriculture Foundation of British Columbia
Funding Title:	Evaluation and Validation of Cow Comfort on B.C. Farms
Funding	Artex Fabricators Ltd.

Agency: Funding Title:	Evaluation and Validation of Cow Comfort on B.C. Farms
Funding Agency: Funding	Clearbrook Grain and Milling
Title:	Evaluation and Validation of Cow Comfort on B.C. Farms
Funding Agency:	Nutritech
Funding Title:	Evaluation and Validation of Cow Comfort on B.C. Farms
Funding Agency:	Western Canada's Genetic Centre
Funding Title:	Evaluation and Validation of Cow Comfort on B.C. Farms
Funding Agency:	Natural Sciences and Engineering Research Council of Canada (NSERC)
Funding Title:	Behavioural strategies in dairy cattle coping with illness
Funding Agency:	Dairy Farmers of Canada
Funding Title:	Building better barns – developing science-based recommendations for cattle housing and management that improve cow comfor
Funding Agency:	Dairy Farmers of Canada
Funding Title:	Feeding behaviour as an early indicator of illness in transition cows
Funding Agency:	Ritchie-Smith Feeds Inc.
Funding Title:	Evaluation and Validation of Cow Comfort on B.C. Farms
Unfunded title:	N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration 102, 6190 Agronomy Road, Vancouver, BC V6T 1Z3 Phone: 604-827-5111 Fax: 604-822-5093