DAIRY CATTLE PREFERENCE FOR DIFFERENT TYPES OF OUTDOOR ACCESS AND THEIR INFLUENCE ON DAIRY CATTLE BEHAVIOUR

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Abstract

Worldwide, the public views pasture as important for dairy cattle to have a good life; dairy cows are highly motivated to access pasture. However, the majority of dairy cows in North America are housed indoors year-round and globally, pasture access is declining. Alternative outdoor areas generally require less space than pasture and may thus be easier to implement. In this dissertation, I examined dairy cow preference for various outdoor areas and how these areas influenced cow behaviour. Chapter 1 reviews how tie- and free-stall housing influence cattle behaviour and how this compares when cows are kept on, or given access to, pasture or another type of outdoor area. Chapter 2 investigates the preference of free-stall housed cows for an outdoor sand pack versus a pasture during the night. When provided simultaneous access to both options, cows spent more time on pasture than on the sand pack (90.5±2.6% versus 0.8±0.5% of the night respectively). When only pasture was available, cows spent $90.0\pm 5.9\%$ of their time outside; this declined to 44.4±6.3% of their time when only a sand pack was available. Chapter 3 investigates cow preference for an outdoor wood-chip pack during summer and winter. Cows spent 25.3±4.3% of their time outside in summer and $1.8\pm0.6\%$ in winter. In summer, cows spent more time on the outdoor pack during the night $(50.0\pm8.4\%)$ than during the day $(3.3\pm1.3\%)$, but this effect was absent in winter (day: $1.7 \pm 0.7\%$; night: $2.1 \pm 1.0\%$). Chapter 4 examines the effect of outdoor space allowance on cow behaviour and preference to be outdoors. During the night, cows spent more time outside with increasing outdoor space; outdoor space did not affect the number of agonistic interactions outside. Chapter 5 investigates the effects of an outdoor pack on oestrus behaviours and showed that access to an outdoor pack facilitated the expression of these behaviours. Overall, dairy cows have a partial preference for an alternative outdoor area but preferred pasture over an

outdoor sand pack during the night, potentially due to a bigger space allowance on pasture or due to the ability to graze; access to an outdoor pack facilitates oestrus behaviours.

Lay Summary

Dairy cows are highly motivated to access pasture. However, in many parts of the world pasture access is declining. Alternative outdoor areas generally require less space and may thus be easier to implement on farms. Therefore, I investigated dairy cow preference for various outdoor areas and how they influenced cow behaviour. Specifically, I investigated cow preference for an outdoor sand pack versus a pasture during the night as well as cow preference for an outdoor woodchip pack during summer and winter. I also examined the effect of outdoor space allowance on cow preference to be outdoors and the effects of an outdoor open pack on oestrus behaviours. In summary, cows have a partial preference for an outdoor open pack, especially during summer nights. When given the option during the night, cows preferred a pasture over an outdoor sand pack. Access to an outdoor pack facilitated the expression of oestrus behaviours.

Preface

All experiments were conducted at the UBC Dairy Education and Research Centre in Agassiz, British Columbia, Canada. The UBC Animal Care Committee approved all experiments and procedures (Protocol A15 – 0082; A14 - 0290).

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Anne-Marieke, June 2019

Dedication

For my family, and especially for opa Meitie, because I know how proud you would have been. And for Bennie, my passion for animal welfare all started with you.

"Grow into your ideals so that life cannot rob you of them"

- Albert Schweizer

Chapter 1: Introduction

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1.1 General introduction

Since the 1950's, farm animal production systems have intensified (Fraser, 2003). One facet of this intensification is a move towards indoor housing systems, which have become the norm for pig and poultry production (Fraser et al., 2001). Societal concerns with such systems emerged more than 50 years ago with the publication of 'Animal machines' (Harrison, 1964) and continue to be discussed (Algers, 2011; von Keyserlingk and Hötzel, 2015). Concerns especially revolve around the lack of outdoor access and the restrictions many indoor systems put on the animals' freedom of movement (Mench et al., 2011) as well as on the ability to perform certain natural behaviours (Miele et al., 2011).

These ethical concerns gave rise to scientific inquires focused on improving the lives of animals. Fraser et al. (1997) put forward three concepts of animal welfare: 1) basic health and functioning, 2) natural living and 3) affective states. The first concept focuses on the functioning of the animals' biological system and emphasizes that animals should be free from injury and disease, and that they should be provided with the basic necessities of life such as food, water and shelter. The concept of natural living emphasizes that animals be provided with an environment in which they can perform behaviours that they are highly motivated to engage in. Lastly, affective states refer to the feelings and emotions experienced by animals and emphasizes the absence of suffering and the presence of positive affective states in animals.

Different stakeholders, driven largely by differences in values, tend to weigh these constructs differently when assessing what aspects are needed for an animal to live a good life. For instance, farmers and veterinarians traditionally emphasize health and biological functioning (e.g. Te Velde et al., 2002; Heleski et al., 2005); whereas, others that are not associated with agriculture, emphasize the natural living aspect (Lassen et al., 2006; Spooner et al., 2014). This disconnect creates challenges for many farmers, particularly as the concept of natural living can seem less intuitive compared to the other concepts and research in this area is lagging (von Keyserlingk and Weary, 2017).

One area of major disconnect between public attitudes and dairy industry practices revolves around access to the outdoors. The public views pasture access as important for dairy cattle (e.g. (Cardoso et al., 2016; Hötzel et al., 2017), but people also value access to natural elements such as fresh air and sunshine and the ability to roam, elements that extend beyond the provision of pasture per se (e.g. Boogaard et al., 2008; Ventura et al., 2016). However, more than 80% of lactating dairy cows in the United States are housed indoors, year-round (USDA, 2016) and pasture access is decreasing in other parts of the world, including Europe (Van Den Pol-Van Dasselaar et al., 2015). Provision of pasture on farms can be difficult, especially on larger farms (Robbins et al., 2016).

In this chapter, I will critically assess the scientific literature to understand how dairy cattle behaviour is influenced by various indoor housing systems, and how this compares to when cows are kept on, or given access to, pasture and other types of outdoor area. Key knowledge gaps regarding the use of alternative outdoor areas will then be explored in the experimental chapters. I begin with an overview of dairy cattle housing systems and discuss the importance of pasture and other types of outdoor access on public perception and measures relevant to dairy cattle welfare. I will then describe how dairy cattle behaviour, specifically feeding, lying, social and oestrus behaviours, are influenced by free-stall and tie-stall housing, two of the most common housing systems, and how this compares to when cows are kept on, or given access to pasture and other types of outdoor area.

1.2 Dairy cattle housing systems

In this section, I provide an overview of pasture and common housing systems used on dairy farms in Europe, Australia, New Zealand, the United States and Canada. This section builds upon the work presented in two recent reviews: one summarized the changes in the global dairy industry affecting dairy cattle health and welfare but did not examine pasture or outdoor access (Barkema et al., 2015) and another focused upon pasture access for dairy cows, but not on alternative types of outdoor access (Charlton and Rutter, 2017).

Europe

Pasture access in Europe varies by country, with Ireland providing 98% and The Netherlands around 70% of their dairy cattle access to pasture; in Greece less than 10% of dairy cows are provided access to pasture (Van Den Pol-Van Dasselaar et al., 2015) (n.b. these figures for pasture usage and other presented below do not distinguish between farms that provide cows a choice to access pasture from a barn, versus being allowed only a single option). Several European countries such as Sweden, Norway and Finland have implemented regulations requiring farms to provide dairy cows access to pasture for specified periods of time (Van Den Pol-Van Dasselaar et al., 2015). For example, in Sweden, dairy cows need to be given pasture access a minimum of 6 hours per day, for 60 - 120 days per year, depending on the region (Jordbruksverket, 2017). These

regulations have been justified based on the assumption that pasture provides the animals with an environment in which they can better express their natural behaviours, such as grazing (Djurskyddslag SFS 2018:1192). In many European countries, the number of farms providing cows with pasture access is declining (Van Den Pol-Van Dasselaar et al., 2015). It is unknown what percentage of farms in Europe use some version of outdoor access other than pasture.

Australia and New Zealand

In 2016, about 99% of Australian farms provided cows pasture access, the large majority (89%) kept cows on pasture year-round; 6% of the farms kept cows on pasture during most of the year but also provided supplementary feed (i.e. partial mixed ration) on an outdoor feed pad; 3% of the farms kept their cows on pasture for less than 9 months per year with a partial mixed ration on an outdoor feed pad, and used some type of indoor housing the rest of the year (Dairy Australia, 2017).

It is thought that more than 99% of dairy farms in New Zealand keep cows on pasture (DairyNZ, personal communication). Approximately one quarter of farms have an off-paddock system (i.e. an area that cows can be kept on during adverse weather conditions, or to reduce feed wastage) available on the farm. Of these farms, 81% have uncovered off-paddock areas; free-stalls were present on 2% of the farms. The lying area comprised at least 80% of the total off-paddock surface area; concrete, gravel and wood-chips were most commonly used as surface material (DairyNZ, 2015).

United States and Canada

Pasture-based dairy farming was once the norm in the United States (Blayney, 2002), but now more than 80% of lactating dairy cows in the US are housed indoors year round, and pasture is used as the primary system for less than 3% of cows (USDA, 2016). Approximately 26% of dairy cows in the US were housed in free-stalls with access to an open/dry lot and 17% were housed in open/dry lots with or without access to a barn or shed (8.8% and 8.3%, respectively) (USDA, 2016). As in many other countries, farm size appears to affect the type of housing used. Small farms (i.e., < 100 cows) more often house cows in a tie stall or stanchion (i.e. a type of housing in which cows are secured to one place), compared to medium (100 - 499 cows) or large $(\geq 500 \text{ cows})$ farms that mainly house their lactating cows in free-stalls without outside access (USDA, 2016). Although the majority of US dairy farms are still relatively small (i.e., in 2012, 78% of all US dairy farms had < 100 cows), almost 50% of all US dairy cows are housed on farms with > 1000 cows (Macdonald et al., 2016). As the percentage lactating cows that have access to pasture decreases with increasing herd sizes (Figure 1), and total milk production continues to shift to larger farms (Macdonald et al., 2016), the proportion of US dairy cows that have access to pasture is likely to continue to decline.

Figure 1. Use of pasture for lactating dairy cows in the US

Percentage of lactating cows in the US that are provided access to pasture, by herd size. Adapted from USDA (USDA, 2016).



Data on pasture access in Canada is limited. Denis-Robichaud et al. (2016) surveyed 832 farms and reported that 24% provided pasture access to their lactating cows for at least part of the year; this practice was more common on tie-stall than free-stall operations (30 versus 16% of farms; 20). No information is available regarding the use of alternative types of outdoor areas in Canada.

Collectively, the available evidence indicates that pasture use differs by continent and country. Pasture usage is generally expected to continue to decline in Europe and North America, driven by increases in farm size (Robbins et al., 2016). With the exception of open lot dairies, some farms provide alternative types of outdoor access, such as bedded open packs or exercise lots (i.e. non-bedded areas with concrete or dirt (i.e. a mixture of earth and manure) as flooring) although numbers on this type of outdoor access in countries other than the US are very limited. It

is also largely unknown what percentage of farms keep their cows on pasture or an alternative type of outdoor area as opposed to providing cows a free choice to access the outdoors or to stay inside some sort of indoor facility or covered area.

Housing systems

To better understand how dairy cattle behaviour changes with outdoor access, it is important to understand how the behaviour of cows is affected when housed indoors. Free-stall and tie-stalls barns are common in the northern hemisphere. The percentage of cows housed in tie-stalls varies greatly among European countries, from as little as 8% of dairy herds in The Netherlands to 78% of herds in Switzerland (Barkema et al., 2015). In Norway the use of tie stalls is being phased out (Simensen et al., 2010). The majority (67%) of the lactating dairy cows in the US are housed in free-stalls; tie stalls and stanchions make up the smallest portion of housing used for lactating dairy cows with 10% of cows housed in these systems (USDA, 2016). No information is available regarding the number of cows kept in different housing systems in Canada, but the Canadian Dairy Information Centre (2018) reported that nearly 75% of Canadian dairy *operations* used tie-stall housing with the remaining farms using free-stall housing. The majority of tie-stalls farms are located in Quebec and Ontario.

1.3 Dairy cattle preference and motivation for outdoor access

Preference testing requires animals to choose between two or more options and can provide insight into an animals' preference for specific aspects of their housing system (Fraser and Matthews, 1997). The 'preferred' option is typically identified as the one that is chosen most often, consumed in the largest quantity, or where the majority of the available time is spent (Kirkden and Pajor, 2006). Motivation testing investigates how hard an animal is willing to work to obtain access to a resource (Duncan, 2005), i.e. a commodity or the opportunity for the animal to engage in a certain behaviour (Kirkden et al., 2003). The stronger the motivation to access a resource, the more important that resource is thought to be for the animal (Dawkins, 1988; Fraser and Matthews, 1997). Hence, welfare is more negatively affected if an animal is denied access to a resource for which it is highly motivated (Fraser and Matthews, 1997).

Several studies have shown that dairy cattle have a partial preference for pasture access (Legrand et al., 2009; Charlton et al., 2011a; von Keyserlingk et al., 2017; Charlton et al., 2013) with cows choosing to spend from 10% (Charlton et al., 2011b) to 72% (Krohn et al., 1992) of their available time on pasture. Preference for pasture is influenced by environmental conditions, with high temperature-humidity index (THI) values (Legrand et al., 2009) and rainfall (Legrand et al., 2009; Charlton et al., 2011b, 2013) decreasing the time spent outside. Cows prefer to spend time on pasture at night rather than during the day (Legrand et al., 2009; Charlton et al., 2011a, 2013), possibly to avoid high solar radiation during the day (Schütz et al., 2009). Several motivation tests have been conducted to investigate preference strength for pasture access. In a study by von Keyserlingk et al. (2017) dairy cows were trained to open a weighted gate to access fresh feed or pasture. Cows pushed as much weight to access pasture as to access fresh feed and worked especially hard to gain pasture access at night. In another study, when walking distance to pasture was increased, cows spent less time on pasture during the day but not at night (Charlton et al., 2013), again indicating that pasture access is especially important during the night. In addition, the quality of the indoor environment may also influence the value of outdoor access for dairy cattle. In a study by Falk et al. (2012) however, cow preference for pasture was not influenced by the number of lying stalls available indoors (24, 16, 8 or 0 per group of 24 cows); cows spent almost 80% of the night on pasture versus close to 40% of the day. Hence, even when provided fewer lying stalls indoors than cows (i.e. overstocked), cows preferred to be indoors for much of the day. More research on how the indoor environment influences dairy cow preference to be outdoors is needed. Given that cow preference for pasture and alternative outdoor areas is affected by many internal and external factors, providing cows a choice may be of particular importance in terms of dairy cattle welfare (e.g. Franks, 2019). In addition, providing animals controllability over their environment likely enhances their welfare (Wiepkema and Koolhaas, 1993).

Little is known about what aspects of pasture are important to dairy cattle. For example, it is not known whether this preference for the outdoors is driven by a desire for more space, cooler air, softer surfaces, grass to graze, or some combination of these and other factors. To obtain more insight into this, I will outline how various dairy cattle behaviours (i.e. lying and standing, feeding, social and oestrus behaviours) are influenced by tie- and free-stall housing as well as by different types of outdoor access, and how providing choice to access the outdoors can affect behaviour.

1.4 The influence of housing systems and different types of outdoor access on dairy cattle behaviour

1.4.1 Lying and standing behaviour

Lying is a highly motivated behaviour in dairy cows, with cows prioritizing lying over feeding behaviour after a period of deprivation of both behaviours (Munksgaard et al., 2005). Heifers appear motivated to lie down for 12 to 13 h per d when housed in a tie stall barn (Jensen et al., 2005); cows trained to push open a weighted gate to access an open deep-bedded lying area worked to maintain a lying time of 13 h per d (Tucker et al., 2018). Cows in free-stall barns typically lie down for 10 to 12 h per d (Cook et al., 2005; Ito et al., 2009; Gomez and Cook, 2010; von Keyserlingk et al., 2012; Charlton et al., 2014). However, there is tremendous variation between free-stall farms in average lying time; Ito et al. (2009) reported farm average lying times between 9.5 and 12.9 h per d and Charlton et al. (2014) reported average farm lying times ranging between 8.7 and 13.2 h per d. In tie-stall housing, lying times also vary from 10 to 15 h per d (Charlton et al., 2016); an average lying time of 12.5 h per d involving 100 Canadian tie-stall farms was reported by Nash et al. (2016). In addition to between-farm variation, cows vary in lying times within farms. For example, von Keyserlingk et al. (2012) found that individual lying times in free-stall herds ranged from 2.8 to 20.5 h per d, and Charlton et al. (2016) found that individual lying times in tie-stalls ranged from 6.3 to 17.9 h per d.

Differences in stall design and flooring can partially explain the between-farm variation (Solano et al., 2016). When compared to a concrete base, cows spend more time lying down in pens with mattresses (Haley et al., 2010) or in tie-stalls with rubber mats (Rushen et al., 2007). The amount of bedding in tie stalls (Tucker et al., 2009) and free-stalls (Tucker and Weary, 2004) also influences lying time, with cows lying more with increased bedding depth. Studies on cubicle design indicate that cows prefer soft (i.e. deep bedded sawdust or sand) rather than hard (i.e. mattresses) surfaces for lying (Tucker et al., 2003) and prefer dry rather than wet bedding (Fregonesi et al., 2007b; Reich et al., 2010). The design of the stall can also affect lying time: cows lie down for less time in stalls with a brisket board (Tucker et al., 2006b) compared to without and in smaller rather than larger stalls (Tucker et al., 2004), suggesting that restrictive stalls are not favoured by cows.

Generally, cows housed on pasture have lower lying times compared to when housed indoors. For example, cows kept on pasture lay down for 10.9 h per d versus 12.3 h per d when

housed in a free-stall barn (Hernandez-Mendo et al., 2007). Other studies reported average daily lying times between 7.5 and 9.5 h per d for cows housed on pasture (Tucker et al., 2008; Sepúlveda-Varas et al., 2014; O'Driscoll et al., 2015). The lower daily lying times on pasture may be a consequence of time spent grazing, but to our knowledge no studies have attempted to disentangle whether indoor housed cows provided access to pasture prefer to graze or to lie for long periods of time. A study by Motupalli et al. (2014) showed that herbage mass on pasture did not have a strong effect on dairy cow preference to access pasture, which may indicate that grazing is not a major driving force for pasture preference. These authors, however, also found a higher feeding rate for cows given access to pasture compared to continuously housed cows, which may indicate a motivation to access pasture. Higher lying times indoors may also be a consequence of boredom; an alternative explanation for the longer lying times reported in free-stalls compared to pasture is that cows are seeking refuge from the concrete standing surfaces elsewhere in the barn; soft, dry standing surfaces are rarely available indoors (Tucker et al., 2006a; Telezhenko et al., 2007). This latter explanation is supported in part by the work of Fregonesi et al. (2004) who housed cows in pens with rubber flooring at the feed bunk and found that cows spent more time standing idle on the rubber flooring at the feed bunk and less time lying down in the stall. A study by Boyle et al. (2007) found no difference in lying time between cows housed in free-stall pens with concrete or rubber flooring, but found that cows housed on concrete stood more in the free-stalls whereas cows in pens with rubber flooring stood more on the rubber flooring at the feed face, again suggesting that cows seek refuge from standing on hard surfaces.

Perching (i.e. standing with only the 2 front feet in the lying stall), often observed in freestall housing, may also be a result of cows looking for a soft place to stand, especially when the placement of the neck rail prevents cows from standing with all four feet in the stall (Fregonesi et

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al., 2009a). When housed in pens with rubber flooring in front of the feed bunk, cows spent less time perching and standing fully in the free-stalls (Tucker et al., 2006a). Taken together, these studies indicate that standing on a soft surface is important for dairy cattle.

Fregonesi et al. (2009b) showed that cows preferred to spend more time both lying and standing fully in an indoor open bedded pack compared to free-stalls, potentially because of the less restrictive environment of the open pack. Despite having lower lying times when housed on pasture, cows given the choice between pasture and a free-stall barn generally chose to lie on pasture rather than indoors (e.g. Ketelaar-De Lauwere et al., 1999; Legrand et al., 2009), except during summer days in warmer climates when cows generally stay indoors (e.g. Falk et al., 2012). Cows are able to engage in a broader range of lying positions when housed on pasture, including lying flat on the side (Krohn and Munksgaard, 1993); the ability to adopt these positions may help explain cow preference for lying on pasture compared to the more restrictive lying environment of free-stalls.

Studies on cow preference and usage of free-stalls compared to an indoor or outdoor open pack are lacking. However, given the available evidence, it can be hypothesised that cows generally prefer open, soft surfaces for lying and standing. An open, bedded outdoor area may provide cows with such an option. I hypothesise that dairy cows would display a partial preference to access such an outdoor area.

1.4.2 Feeding behaviour

Dairy cattle are ruminants and able to utilize high roughage diets, but to maintain milk production and minimize body condition loss (Kolver and Muller, 1998), many dairy cattle are also fed grain (sometimes in the form of a total mixed ration, TMR; Eastridge, 2006; DeVries et al., 2007). Ration formulation may vary based on the nutritional demands of cows in relation to their stage of lactation (see NRC., 2001). On average, milk production increases when the diet is supplemented with grain (Bargo et al., 2002; White et al., 2002), and the perceived production benefits of feeding a mixed ration may be a major reason why many cows are no longer kept on pasture (USDA, 2016).

It is important to distinguish between choice and forced outdoor systems. When cows were provided a choice between free-stall housing and pasture, they maintained much of their TMR intake, and increased their feeding rate as compared to when they were confined in the free-stall barn (Legrand et al., 2009). Cows can also maintain their intake (and milk production) when kept outdoors at night and indoors during the day, relative to cows kept permanently indoors (Chapinal et al., 2010).

Feeding and rumination times of cows fed a TMR averaged 4.5 and 7.3 h per d, respectively (reviewed by White et al., 2017); whereas, feral cattle spend can spend up to twice as much time grazing (6.8 - 13.0 h) and about 1.5-fold more time ruminating each day (4.7 - 10.2 h) (reviewed by: Kilgour, 2012). Some have speculated that cows may experience frustrated feeding behaviour given the decreased time spent orally manipulating and processing the TMR compared to when grazing (Redbo, 1992; Redbo and Nordblad, 1997). Given that grazing is a natural behaviour for cows, and highly important for survival (Kilgour, 2012), it is likely that cows are highly motivated to graze. Frustrated feeding behaviour is associated with the development of stereotypic and other abnormal behaviours in many animal species (e.g. pigs: Jensen et al., 2010, giraffe: Koene, 1999, chimpanzee: Baker and Easley, 1996; horses: Willard et al., 1977).

Stereotypic behaviours often resemble the behaviour that is thwarted (Jensen, 1988). When grazing, cattle roll their tongue around the grass to ingest it; this behaviour resembles tongue

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playing or tongue rolling (i.e. 'twisting and twirling with the tongue, either inside or outside the open mouth', (Krohn, 1994), one of the most common stereotypies in cattle. In experimental settings, tongue rolling was not observed on pasture (Redbo, 1990, 1992, 1993). Thus, the method of feed ingestion may be as important for animals as the goal itself (i.e. ingesting feed). Interestingly, in mountain breeds such as Brown Swiss and Simmental, tongue rolling is more prevalent (Sambraus, 1985). The reason for this is unknown. Jerseys also seem to be especially orally motivated, showing a higher frequency of cross-suckling than Danish Red or Holstein Friesian calves (Nielsen et al., 2008). The lower prevalence of tongue rolling behaviour in other breeds does not necessarily indicate that they are less motivated to graze or to obtain roughage.

As pasture provides cattle with roughage, grazing is confounded with roughage consumption. As described by Beauchemin (2018), cows fed a TMR use their lips to ingest feed, as opposed to their tongue to ingest long-stemmed forage or when grazing grass (Redbo, 1990). Given that prior experience may play an important role in determining the preference for pasture (Charlton and Rutter, 2017), and that grazing behaviour may be learned (Costa et al., 2016; Charlton and Rutter, 2017), grazing itself may not be the only factor influencing the preference for pasture access. Research in this area is again limited, but the development and frequency of stereotypies has been linked with feeding low amounts of roughage (Redbo and Nordblad, 1997). Calves preferred long over chopped hay in a study of Webb et al. (2014), and work on beef cows found that they were highly motivated to obtain roughage, especially when kept on a low-roughage diet (Van Os et al., 2018). Collectively, these results indicate that access to roughage and the manipulation of feed are important to cattle.

The time of day that cows spend feeding indoors is mainly determined by the time of fresh feed delivery (DeVries et al., 2005). On pasture, cows feed mainly during the day, with intense

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grazing bouts at dawn and dusk (Ruckebusch and Bueno, 1978; Gregorini, 2012). Cows kept at pasture often show synchronized feeding (Rook and Huckle, 1995) and lying behaviour (Stoye et al., 2012), which is thought to be positive for their welfare, perhaps especially so for more subordinate cows (Metz, 1983).

Given that milk production per cow has more than doubled in the last 40 years (Oltenacu and Broom, 2010), selection for milk yield may cause high producing dairy cows to be highly motivated to consume a high energy TMR ration, which is normally provided indoors. Given the selection for high milk production and the correspondingly high energy requirements, it has been questioned if certain dairy genotypes are suitable to be housed exclusively on pasture (Dillon et al., 2006). Interestingly, cows that had free access to pasture had a higher milk production than cows continuously housed in a study by Motupalli et al. (2014). Feeding cows a TMR without providing cows the opportunity to graze may result in frustrated feeding behaviour. We encourage research to disentangle the importance of grazing and roughage provision for dairy cattle. Given that cows are unable to perform grazing behaviour in alternative outdoor systems, understanding the importance of grazing for dairy cattle welfare will also provide more insight into the acceptability of providing cows these alternatives.

1.4.3 Social behaviour

Social behaviour includes positive and negative (or agonistic) interactions. Positive interactions in cows have not been studied extensively but there is some evidence that allogrooming (i.e. social licking) is important (Endres and Barberg, 2007; Val-Laillet et al., 2009). In contrast, agonistic interactions between cows have been studied extensively and consist of multiple forms of aggressive behaviour, such as displacements, pushes and head butts (Krohn,

1994). Housing is thought to play an important role in the frequency and display of social interactions between cows (Tresoldi et al., 2015).

By design, tie-stalls reduce aggressive behaviour between cows (Popescu et al., 2013; Rushen, 2017; Beaver et al., 2019), but they also limit the animals' ability to engage in allogrooming. Krohn (1994) showed that the frequency, but not the duration, of social licking was decreased in cows housed in tie stalls compared to loose-housed cows

In free-stall housing, competition for access to key resources such as feeding and lying areas may pose challenges. Stocking density can influence the social behaviour of dairy cattle. When given a choice, cows prefer to have greater inter cow distances than what is normally available in indoor systems (Kondo et al., 1989; DeVries et al., 2004). Heifers housed on a woodchip pack and provided an individual space allowance of 8 m₂ on the pack and 6 m₂ on the concrete feeding area, had a higher overall frequency of play and locomotor behaviour than heifers housed inside a free-stall pen provided 5.3 m2/heifer (Boyle et al., 2008). They also had a higher frequency of allogrooming, but no difference was found in the frequency of agonistic interactions. Tresoldi et al. (2015) investigated the effects of housing type on social behaviour in dairy heifers housed in either a free-stall barn or kept on pasture. When housed in free-stalls, heifers exhibited a 4-fold increase in the number of social interactions (allogrooming as well as agonistic interactions) compared to when housed on pasture, but the ratio of positive to negative interactions was the same in the two environments. Less space was available indoors than on pasture, leading the authors to suggest that the higher number of social interactions observed indoors was a consequence of a higher stocking density. It is well known that increased stocking density leads to a higher level of competition for access to the feeding area in free-stall housed dairy cattle (e.g. Huzzey et al., 2006; Krawczel et al., 2012).

Another challenge for dairy cattle is regroupings. Under natural conditions, cattle live in socially stable groups of varying ages (Reinhardt and Reinhardt, 1981). Regrouping is stressful and affects the social hierarchy (Bøe and Færevik, 2003). Cows use physical and non-physical interactions to re-establish social relationships in a group (Kondo and Hurnik, 1990); and these effects are greatest when less space per cow is provided (Talebi et al., 2014). The acute effects of regrouping, such increased agonistic interactions, can last up to 3 days (von Keyserlingk et al., 2008).

Some studies have investigated the effect of space allowance on the behaviour of cows. Fregonesi and Leaver (2002) examined space allowances of 4.5 or 9 m₂ per cow in a straw yard system; no effects on lying time or agonistic interactions were noted. Schütz et al. (2015) reported that a minimum of 6 m₂ of space allowance per cow was needed on a rubber mat during an 18 h stand off period (i.e. a period of wet weather during which cows are removed from pasture) to maintain daily lying times similar to that observed when cows were housed on pasture. When cows were provided less space (3 or 4.5 m₂ per cow), the reduction in both lying time and lying bouts was thought to be due to increased agonistic behaviour.

Given that increases in space allowance result in reduced interactions between cows, it follows that providing cows with an additional outdoor space will result in a decline in social interactions. However, there has been little experimental work looking at how much space cows require when provided outdoor access. The Canadian Dairy Cattle Code of Practice (NFACC, 2009) states that resting areas in bedded-pack pens must provide 11 m² per mature cow, but no justification is provided for this number. New research is required to investigate the space requirements of individual cows when provided different forms of outdoor access. As social rank may play an important role in the preference of dairy cows for certain environments, studies should

include social rank when investigating cow preference. In addition, the effect of the choice to go outdoors on social interactions should be investigated, especially on outdoor areas other than pasture that provide less space per individual cow.

1.4.4 Oestrus behaviour

The oestrus cycle in dairy cows is, on average, 21 days in length (Savio et al., 1990), with oestrus behaviour expressed between 2 and 24 hours (Forde et al., 2011). Oestrus behaviour in dairy cows can be divided into primary (i.e. standing to be mounted) and secondary signs (i.e. anogenital sniffing, chin resting, successful and unsuccessful mounts) (Sveberg et al., 2011).

Cows housed in tie-stalls have little opportunity to express oestrus behaviours (Felton et al., 2012). Kiddy (1977) showed an approximate 3-fold increase in activity in cows housed in tie-stalls at the time of oestrus as measured by pedometers. Other studies reported increased activity, such as the number of steps taken, in tied cows (Redden et al., 1993; Kennedy and Ingalls, 1995) and in heifers (Sakaguchi et al., 2007) around the time of oestrus. However, all studies except for Sakaguchi et al. (2007) provided animals with periods in an exercise yard (Redden et al., 1993; Kennedy et al., 2011) or a holding pen before milking (Kiddy, 1977). Indeed, Redden et al. (1993) reported that activity increases were only observed during the daytime, when cows were given an exercise period, and not at night when cows were confined. Felton et al. (2012) continuously tied cows, and found no increase in activity during oestrus. De Silva et al. (1981) showed that, when placed onto a dirt lot, cows housed in stanchion barns exhibited more mounts per hour (11.2 ± 9.0) than cows housed in a free-stall (6.5 ± 6.8) or on pasture (5.4 ± 2.9). Together, these results indicate that continuously tying cows inhibits their expression of oestrus, perhaps explaining why a higher percentage of tie-stall farms use timed artificial insemination (i.e. insemination without the need

for oestrus detection using injections with hormones to synchronize oestrus; Souza et al. (2009)) as the main reproductive management practice compared to free-stall farms (Denis-Robichaud et al., 2016).

Free-stall housing allows cows greater freedom of movement. However, systems where cows are continuously on concrete flooring (representing the vast majority of US dairy operations; USDA, 2016) pose a challenge for oestrus expression. Cows housed in free-stall barns with concrete flooring have fewer standing oestrus events (Palmer et al., 2010) and a lower frequency of standing to be mounted compared to cows housed on pasture (Palmer et al., 2012). Similar results were found comparing concrete with other types of flooring; for example, cows had a lower duration of oestrus as well as a lower frequency of mounting and standing to be mounted when kept on concrete compared to dirt flooring (Britt et al., 1986). The effects of rubber flooring are variable; cows housed on rubber mats showed a higher frequency of mounting than when housed on concrete (Platz et al., 2008), but no beneficial effects on oestrus behaviour of rubber over concrete flooring were found by Boyle et al. (2007). Differences in rubber quality, especially with regard to friction, may explain this difference (Phillips and Morris, 2001, 2002).

Vailes and Britt (1990) suggested that cows may feel unsure of their footing on concrete and are therefore less inclined to perform oestrus behaviours. Concrete flooring has been linked with more slipping during mounting compared to pasture (Palmer et al., 2010) or rubber flooring (Platz et al., 2008). In the latter study, 19 out of 23 mounts on a concrete floor were accompanied with collapsing or slipping. In another study, cows housed in a straw yard had a lower number of unsuccessful mounting attempts compared to cows housed in a free-stall (Phillips and Schofield, 1994), possibly because the straw flooring provided them with a less slippery flooring. Indeed, when given a choice between concrete and dirt, cows in oestrus spent more time on dirt than on concrete flooring and preferred to mount other cows that were in oestrus on dirt rather than on concrete flooring (Vailes and Britt, 1990). However, the latter study was conducted with individual cows that were given 30 minutes to interact with two tied cows, one on concrete and one on dirt; to our knowledge no research has examined preferences for different types of flooring during oestrus in dairy cows housed under commercial conditions.

Concrete flooring can also increase the risk of lameness in dairy cows (Hernandez-Mendo et al., 2007; Adams et al., 2017). Lame cows may be less inclined to engage in oestrus behaviours, especially if the flooring contributes to their pain (Palmer et al., 2010). Lame cows have lower behavioural oestrus expression than non-lame cows (e.g. Walker et al., 2008). In addition, falling and slipping when mounting can increase the risk of trauma and therefore lameness.

Based on these results, it seems that housing systems with softer, high traction flooring such as pasture or dirt, facilitate the expression of oestrus behaviour in dairy cows. Hence, access to an outdoor area with better footing than is provided by concrete may be especially beneficial to cows in oestrus.

1.5 Thesis aims

Pasture can provide cows with an open area and a soft surface that allows the expression of grazing and facilitates the expression of lying, standing and oestrus behaviours. In addition, keeping cows on pasture decreases negative social interactions between cows, potentially because cows on pasture engage in fewer encounters as compared to when housed indoors. When pasture access is not feasible, one alternative is to provide cows access to an outdoor open bedded pack. Given the lack of information regarding the use of outdoor packs in dairy farming, the overall aim
of my thesis was to examine dairy cow preference for various outdoor areas and how access to these areas influences their behaviour. More specifically, my aims were to investigate:

- 1) The preference of cows for different types of outdoor access and how access to various outdoor areas influences lying, standing and perching behaviour (Chapter 2),
- 2) The preference of cows for access to an outdoor-bedded pack during summer and winter and how this access influences lying, standing and perching behaviour (Chapter 3),
- Effect of outdoor open pack space allowance on the behavior of free-stall housed dairy cows (Chapter 4) and
- The effects of an outdoor-bedded pack on the expression of oestrus behaviours in cows (Chapter 5).

Chapter 2: Dairy cow preference for different types of outdoor access

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2.1 Introduction

Pasture access provides certain benefits to dairy cows (reviewed by: Charlton and Rutter., 2017), including increased opportunities to express natural behaviour such as grazing and exploring. Providing cows access to pasture can also be positive for udder (Washburn et al., 2002), foot and leg health (Haskell et al., 2006; Olmos et al., 2009).

Free-stall housed cows spend different portions of their time outside when given access to pasture, with the percentage of time spent outside varying across studies from 72% (Krohn et al., 1992) to as little as 10% (Charlton et al. 2011a). One reason for this variation may be that cows in different studies varied in their experience with pasture. Experience can influence preference (Kirkden and Pajor, 2006), with animals often preferring environments that they are familiar with (Fraser and Matthews, 1997). This may help explain why the cows in the study of Charlton et al. (2011a), that had limited pasture experience, spent more time indoors.

Preferences of animals can be complex (Fraser and Matthews, 1997). For example, cows may prefer different environments to engage in different behaviours. They may prefer one environment for feeding, but another for socializing. Many factors influence the preference of dairy cows for pasture access. An important factor that influences preference for pasture is the weather (e.g. Legrand et al., 2009; Charlton et al., 2011a; b). Cows spend more time on pasture at night (Charlton et al., 2011a, 2013; Motupalli et al., 2014), especially when ambient temperatures

during the day are high (Legrand et al., 2009). It has also been shown that distance to pasture affects its use during the day but not during the night, which is consistent with a higher motivation of cows to access pasture during the night (Charlton et al., 2013; Motupalli et al., 2014).

Despite the clear benefits of pasture access for dairy cattle, it is often difficult to implement pasture access on dairy farms. Outdoor areas other than a pasture may be more practical to implement on some farms, as the space requirements are normally lower than for a pasture. However, little is known about what aspects of outdoor access are important to dairy cattle (Charlton and Rutter, 2017). For instance, are cows motivated specifically to graze? Or is their preference driven by preferences for alternate lying and standing surfaces not available indoors?

To our knowledge, no work has attempted to disentangle whether free-stall housed cows prefer to access a pasture versus some other outdoor area, particularly during the night when cows show the strongest motivation for outdoor access (von Keyserlingk et al., 2017). In addition, although some work has shown welfare benefits of exercise in an outdoor pack (Loberg et al., 2004; Regula et al., 2004), no work has investigated if the behaviour of cows while in the barn changes when the cows also have access to the outdoors. As changes in flooring (Fregonesi et al., 2004) and cubicle design (Bernardi et al., 2009) can influence the standing, lying and perching behaviour (standing with the 2 front hooves in the stall) of cows, the provision of outdoor access may also lead to changes in behaviour of cows when inside their normal free-stall housing.

Cows prefer to lie on pasture as opposed to in free-stalls when environmental conditions are favourable (Legrand et al., 2009; Falk et al., 2012), probably because pasture provides cows with a less restricted environment than any type of loose housing environment (Krohn and Munksgaard, 1993; Charlton and Rutter, 2017). A soft outdoor pack can provide cows with some of the same benefits as pasture, as it allows cows to stand, walk and lie down without having to

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navigate the confines of a free-stall; indeed, when given a choice between the free-stalls and an indoor open sand pack, cows spent more time lying and standing fully (i.e. with 4 feet) in the pack than in the free-stalls (Fregonesi et al., 2009b). In addition, cows spent more time standing outside of the stall (typically on wet concrete surfaces) and more time perching with their front legs on the bedded surface when in free-stall cubicles versus the open pack (Fregonesi et al., 2009b), behaviours that increase the risk of lameness (Bernardi et al., 2009).

The primary objective of this experiment was to determine the preference of lactating dairy cows for pasture versus an outdoor sand pack during the night. Our second objective was to determine whether feeding and perching behaviour inside the barn changed when cows were provided outdoor access. A third objective was to investigate how lying behaviour was affected by providing cows access to different outdoor areas.

2.2 Materials and methods

2.2.1 Cows and treatment

This experiment was carried out at the University of British Columbia Dairy Education and Research Centre (Agassiz, BC, Canada) and took place between August and October 2015. This experiment and all procedures were approved by the University of British Columbia Animal Care Committee (Protocol A15 - 0082).

We used 96 pregnant Holstein cows that were assigned to 8 groups (12 cows/group) [parity $(2.5 \pm 0.2; \text{Mean} \pm \text{SD})$, Days In Milk (DIM) (243 ± 17) , projected 305-d milk production (10,937 $\pm 448 \text{ kg})$, Body Condition Score (BCS) $(3.4 \pm 0.1; \text{ range: } 2.5 - 4.5)$ and gait score (2.0 $\pm 0.1;$ range: 1-3)]. Two experienced observers assessed the BCS and gate score of each cow. BCS was assessed using a 5-point scale (1 = severely under condition, 5 = severe over condition) with

quarter point increments following Edmonson et al. (1989). Gait scoring was done using a 5-point scale (1 = healthy, 5 = severely lame) following Flower and Weary (2006). Severely lame cows (gait score 4 and 5) were not included in the experiment. The majority of cows had previously been kept on pasture for varying periods as heifers and some had also been kept on pasture during previous dry periods.

Two groups were tested simultaneously. Each group was housed in 1 of 2 experimental pens for at least 14 d. After regrouping, animals were given at least 3 d to allow for the social behaviour to stabilize (see von Keyserlingk et al., 2008). Groups were kept in the free-stall barn for 2 additional days to allow for baseline observations (Baseline phase). All animals had previous experience with sand bedding as they are kept on sand bedded free-stalls. Animals were given access to the sand pack and the pasture on alternate days for approximately 24 h each (i.e. from 11:00 h until morning milking the following day) before data collection began. To ensure that cows were familiar with both outdoor areas during this habituation period they were moved outside during these experience days at 15:00 h, 20:00 h, 22:00 h and 06:00 h, if not already outdoors.

The data collection part of this experiment consisted of 2 parts. The first followed immediately after the habituation phase. Cows were provided access to either the pasture (Pasture phase) or the sand pack (Sand phase) for 2 nights each. Nights were defined as the time between 2000 h until the next morning milking; from morning milking until 2000 h cows were confined to the free-stall barn. Order of access to the different outdoor areas was balanced among the groups. On the first day of both the Pasture phase and the Sand phase all animals were forced outside. For the final part of the experiment groups were given access to both outdoor areas for 3 successive nights (Choice phase). The third day of the Choice phase consisted of the night time only.

2.2.2 Housing, management and diet

The 2 experimental pens (Figure 2.1) were located in a mechanically ventilated (72" Artex Storm Fan, Artex Barn Solutions, Abbotsford, BC) wooden frame free-stall barn (42 x 93 m) with a north-south orientation and curtained sidewalls. Each pen (7.3 x 13.5 m) consisted of 12 lying stalls (2.4 x 1.2 m), configured in 3 rows of 4 stalls filled with \pm 40 cm of washed river sand. Stalls were divided by Dutch-style partitions (Y2K stall dividers, Artex Barn Solutions, Abbotsford, BC) spaced 1.2 m wide centre-to-centre with the neck rail placed 1.3 m above the stall surface and 1.4 m from the inside of the rear curb. The 0.2 m high brisket board was placed 1.8 m from the inside of the rear curb that measured 0.2 m high from the alley floor. The concrete alleys were cleaned 6 times daily with an automated scraper; cross-over alleys were manually cleaned twice per day. Each pen had a headlock feed barrier with 12 headlocks per pen, 60 cm wide centre-to-centre.

Cows were fed a Total Mixed Ration (TMR) formulated following the National Research Council (NRC) guidelines (NRC, 2001) to meet or exceed the requirements of a 659 kg Holstein producing 34 kg/d of milk. The TMR consisting of 33 % corn silage, 48 % concentrate mash, 14 % grass silage and 5 % alfalfa hay on a Dry Matter (DM) basis was fed inside during the complete experimental period and was available *ad libitum*. Fresh feed delivery took place between 0530 and 0630 h for 1 group and between 0630 and 0730 h for the other group. Feed was pushed up at approximately 1100 and 2230 h and orts were taken away at approximately 0530 h. Animals had *ad libitum* access to fresh water provided from a self-filling water trough located on the cross over alley. Each outdoor area also contained 1 self-filling water trough.

Animals were milked twice daily in a double-12 parallel milking parlour between 0730 and 0830 h in the morning and between 1730 and 1830 h in the afternoon. If animals were outside at the time of morning milking they were moved directly to the parlour.

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Figure 2.1 Schematic of experimental areas

Overview of the experimental areas used to test the preferences of lactating dairy cows for different types of outdoor access.



2.2.3 Outdoor areas

Both the pasture and sand pack (Figure 2.1) were lined with electric fencing. Outdoor paths were covered with rubber mats. The sand pack was covered with approximately 15 cm washed river sand and measured 144 m² (12 x 12 m). Each pasture plot was 21,000 m₂ (350 x 60 m). The pasture, planted in April 2015, consisted of 10% orchard grass (*Dactylis glomerata L*.), 43% tall

Fescue (*Festuca arundinacea*), 43% Festulolium (*Festulolium pabulare*) and 4% Annual ryegrass (*Lolium multiflorum*). Samples of the pasture were taken at the beginning of October (n = 5) and November (n = 8) to determine pasture quality. Approximately 40% of the field furthest from the barn was mowed at the end of September to allow re-growth and harvest of the grass. As the pasture plot was significantly large, providing 1750 m² per cow, it is unlikely that this would have affected pasture use. All feed samples were dried at 60 °C for a total of 48 h to determine DM content. Dried samples were ground and sent for nutritional analysis (A&L Laboratories Inc., London, ON). During the experiment, pasture mass averaged (\pm SD) 1.11 \pm 0.5 kg/m² of fresh matter, 17.8 \pm 3.0 % DM and (expressed as % DM) 22.5 \pm 2.3 % Crude Protein, 57.3 \pm 1.9 % Neutral Detergent Fibre and 33.2 \pm 3.7 % Acid Detergent Fibre.

2.2.4 Behavioural measures

The behaviour of the cows was recorded using video. Cameras were placed (Panasonic WV-CW504SP outdoor video camera, Sandpiper Technologies Inc., Manteca, CA) 6 m above the entrance of the barn, 8 m above the indoor alley that connected the outdoor areas with the experimental pens and 8 m above each pen to provide an overview of the lying area. Above each experimental pen another camera (Panasonic WVCP-470, Panasonic Corporation of North America, NJ, USA) was placed 6 m above the feed bunk. All recordings were stored using a GeoVision 1480 digital recorder (USA Vision Systems, Irvine, CA). Infra-red lights (BR38 Red Incandescent Flood Light 100 W, Globe Electric company INC., Montréal, Canada) were placed adjacent to each camera to facilitate the observation of the cows during the night. Each cow received a unique symbol on her back made with hair dye to facilitate individual recognition of animals. Cows were scored as feeding and perching using 5-min scan sampling. Feeding was

defined as the cow having her head completely through the headlock and perching was defined as the cow standing only with her 2 front feet in the lying stall. Location of the animals (i.e. in the pen, pasture, or sand pack, or in the indoor or outdoor alley) was scored using 5-min scan sampling. Grazing behaviour was not recorded in this experiment.

HOBO data loggers (HOBO Pendant G, Onset, Cape Cod, MA) were used to quantify lying times (UBC AWP, 2013). The data loggers were programmed to record the posture of the cow, i.e. lying or standing, in 1-min intervals. The logger was attached to 1 of the cows' rear legs before the beginning of the Baseline phase and was removed after the experimental period. Loggers were attached and removed in the milking parlour.

2.2.5 Climatic measures

For each experimental day, hourly mean air temperature, maximum relative humidity, mean wind speed and total precipitation were recorded by the Environment Canada weather station in Agassiz, located 400 m from the UBC Dairy Education and Research Centre. Temperature, relative humidity and wind speed during the night-time (i.e. from 8 pm until 8 am) averaged (\pm SD) 12.6 \pm 3.0 (range: 3.8 – 20.5) °C, 81.7 \pm 15.4 (range: 41.8 – 98.1) % and 1.6 \pm 1.6 range: 0 – 8.8) m/s. It rained on 9 out of 28 experimental days. Rainfall averaged 0.11 \pm 0.33 (range 0 – 2.4) mm on days that it rained. Temperature-humidity index was calculated as: THI = (1.8T + 32) – [(0.55 – 0.0055 RH) x (1.8T – 26)] with T = air temperature (°C) and RH = relative humidity (%) (Ravagnolo et al., 2000) and averaged (\pm SD) 55.1 \pm 4.8 (range: 40.0 – 66.8).

2.2.6 Statistical analyses

During the experiment, 1 cow was identified as lame and 2 were diagnosed with an udder injury; all 3 were excluded from all data analyses. Two other cows came into heat; data collected on the days of oestrus were discarded from all cows in the pen. Only one of these cows was removed from the group, as she showed signs of oestrus on the day that data collection took place. One group was excluded from the analysis of feeding behaviour because of a malfunction of the headlocks. Of this group, 1 animal was excluded for all analysis and 3 others were excluded for 1 day (2 animals) and 2 days (1 animal) as they were locked in the headlocks on these days for > 3 consecutive hours. For 2 groups, the last 24 h of data collection were excluded from all analysis, because cows were accidentally given access to the outdoor areas during part of the day.

Data were summarized by group (i.e. each group represented the average of 12 cows) and phase. All analyses were performed considering group (n = 8) as the experimental unit. Data were scrutinized using PROC UNIVARIATE in SAS for normality and homogeneity (version 9.4, SAS Institute, Institute Inc., Cary, NC). Data were separated into day (from return from morning milking until 20:00 h) and night (20:00 h until morning milking) periods. Analyses were performed using PROC MIXED in SAS. To test preference for the various locations (i.e. pen, pasture or sand pack), the MIXED model included phase as fixed effect and group as random effect. All *P*-values were corrected using a Bonferroni correction.

Weather conditions (relative humidity, air temperature, wind speed, precipitation and THI) were included as covariates, but as they never had a significant effect on the amount of time cows spent outside, results are not reported elsewhere. However, when plotted, it appeared that high hourly rainfall (i.e. \geq 1.4 mm per hour) reduced the time spent outside. Therefore, the 2 days with \geq 1.4 mm of rain were removed from all analyses.

Differences in feeding, perching and lying behaviour were analysed using phase as a fixed effect in the model and group as a random effect. For the analysis of feeding, perching as well as lying behaviour over the 24-h period we used a contrast statement in the MIXED model to test: 1) if behaviours differed between the Pasture phase and Sand phase and, if this was not significant, 2) if the Pasture and Sand phases combined differed from the Baseline phase. All models were run with a Bonferroni correction. Significance was declared when P < 0.05.

2.3 Results

2.3.1 Preference

When free-stall housed cows were provided access to only pasture (Pasture phase) they spent 90.0 \pm 5.9 % (range 75.6 – 100%) of the available time outside; in contrast, cows spent about 44.4 \pm 6.3 % (range: 9.8 – 72.4%) of their time outside when provided access to only the sand pack (Sand phase) (Figure 2.2; F_{1.6} = 29.03, *P* < 0.01). When the cows were provided simultaneous access to both pasture and the outdoor sand pack (Choice phase) they spent 90.5 \pm 2.6 % (range: 80.0 – 99.4%) of the time available on pasture and 0.8 \pm 0.5 % (range: 0 – 4.5%) of the time on the sand pack.

Figure 2.2 Time cows spent outside during the night when given a free choice to access different outdoor areas

Mean (\pm SEM; %) time groups of lactating dairy cows (n = 8 groups) spent outside (grey bar) and inside the free-stall barn (white bar) during the night (20:00 - ~ 8:00) when provided free choice between the free-stall barn and pasture and between the free-stall barn and pack.



2.3.2 Behaviour in the free-stall barn

Time spent feeding indoors during the day (i.e. from morning milking until 20:00 h) did not change when comparing Baseline, Pasture and Sand phases (F_{2,11} = 0.25, P = 0.7812; Table 2.1). Cows spent less time perching in the stall during the day during both the Pasture and Sand phase compared the Baseline phase (F_{2,13} = 18.06, P < 0.001). During the 24-h period, time spent feeding indoors was highest during the Baseline phase, intermediate in the Sand phase and lowest in the Pasture phase (F_{2,11} = 92.13, P < 0.001). The same pattern was found for perching behaviour (F_{2,13} = 83.35, P < 0.001).

Table 2.1 Mean time spent on various behaviours

Mean (\pm SEM; %) of total time spent feeding and perching (standing with the 2 front feet in the lying stall) in the free-stall barn during the day (~ 8:00 to 20:00 h), lying on pasture during the night (20:00 to ~ 8:00 h) and over 24-h (n = 8 groups of lactating dairy cows).

Behaviour		Phase		
	Baseline	Pasture	Sand	Р
Feeding – day	23.8 ± 1.0	23.4 ± 1.0	24.1 ± 1.0	0.781
Perching - day	$5.0 {\rm a} \pm 0.4$	$3.3 \texttt{b} \pm 0.4$	$3.7 \text{b} \pm 0.4$	< 0.001
Feeding – 24 h	$18.2a \pm 0.4$	$12.8c \pm 0.4$	$16.3b \pm 0.4$	< 0.001
Perching – 24 h	6.11 a ± 0.4	1.72 c ± 0.4	2.88 b ± 0.4	< 0.001
Lying – 24 h	$57.2b \pm 1.3$	$52.3a \pm 1.3$	59.0 b ± 1.3	< 0.01

2.3.3 Lying behaviour

Over the 24-h period, lying time varied between the Baseline, Sand and Pasture phases $(F_{2,13} = 11.52, P < 0.01)$ (Table 2.1); this was driven by lower lying times during the Pasture phase compared to the Sand and Baseline phase; the lying time in the Baseline phase did not differ from the Sand phase.

The percentage time cows spent lying when outside was not different in the Sand phase $(55.4 \pm 7.9 \%)$ vs. the Pasture phase $(52.0 \pm 7.4 \%)$ (F_{1,6} = 0.14, *P* = 0.718).

2.4 Discussion

When allowed free access to pasture during the night in this study, cows spent around 90% of their time outside. Other authors (Krohn et al., 1992; Charlton et al., 2011a; Motupalli et al., 2014) found that cows spent around 70% of their total time outside when given a choice between pasture and a free-stall barn, but this number is a combination for day and night and in the latter 2 referenced studies pasture use was highest at night. Previous work (Legrand et al., 2009; Falk et al., 2012) found that cows spent about 80 - 90% of their time outside at night and tended to stay indoors during the day. Cattle are sensitive to heat stress (Blackshaw and Blackshaw, 1994),

partially explaining why cows spend more time outside during the night. In addition, it appears that cows are specifically motivated to avoid solar radiation, an important feature in the design of shade for dairy cows (Schütz et al., 2009). Thus, avoiding direct sunlight and the consequences in terms of radiant heat may be a reason why outdoor access is especially preferred at night, at least during the summer months. Cattle also have been shown to avoid rain (Legrand et al., 2009; Charlton et al., 2011b, 2013). As the difference in time spent outside on the sand pack versus the pasture was very large, it was not possible to reliably test the effect of weather on the time spent outside on the different outdoor areas. However, readers should also consider that the weather conditions under which this study was conducted were typical for the lower Fraser Valley region of British Columbia. During this study, the outside air temperature ranged from 3.8 - 20.5 °C, a range of temperatures that falls well within the lower (Hamada, 1971) and upper (Berman et al., 1985) critical temperature range for dairy cattle. In addition, rainfall affected the time animals spent outside only on a few days.

Cows that were provided access to an outdoor sand pack used this option, but only spent about 44% of their time outdoors. When allowed access to both outdoor options, cows showed a preference to access pasture over the sand pack. This preference may have been due to the greater available outdoor space on pasture versus the sand pack. By design the space provided was different between the 2 outdoor options as we tested the sand pack and pasture options using space allowances consistent with what would be practical on commercial dairy farms. Future experimental work could examine the role of space independent of surface. In addition, future studies should also investigate how much space should be recommended per individual free-stall housed cow on an outdoor open pack. The fact that cows could graze while on the pasture may also explain the preference for this option compared to the sand pack, particularly if grazing is a rewarding activity for dairy cows. Little is known about the motivation of cattle to graze (Charlton and Rutter, 2017) and we encourage work in this area, as the inability to graze may be an important constraint in the development of alternative forms of outdoor access for cattle.

The cows used in this experiment had varying degrees of previous experience with pasture, but the outdoor sand pack was novel. Cows were provided a habituation period for both options, but this period may have been inadequate for the sand pack. Previous work has shown that cows may require long adaptation periods to overcome initial preferences (Tucker et al., 2003). Therefore, the amount of time cows spend in the sand pack may have been higher if a longer habituation period was given.

Feeding time inside the barn during the day was not affected when cows had the choice to be outdoors during the night to either a pasture or sand pack. This result is in line with the findings of Chapinal et al. (2010) who showed that overnight pasture housing did not decrease TMR intake. However, taken over a 24-h period, feeding time was lowest in the pasture phase, intermediate in the sand phase and highest in the baseline phase. It is possible that cows increased their feeding rate, as was observed by Legrand et al. (2009), to maximize the time spent outdoors under favourable weather conditions.

In line with previous work (Hernandez-Mendo et al., 2007; Legrand et al., 2009; Charlton et al., 2013), our study found the lowest lying times when cows were given access to pasture. Legrand et al. (2009) suggested that lower lying times on pasture might be due to time spent grazing on the pasture. Given that the lowest feeding times also occurred during the Pasture phase we speculate that cows spent a considerable amount of time grazing during this phase. However,

as we did not take any observations when the cows were on pasture, we are unable to confirm this. Future work of this nature should consider equipping the animals with automated grazing monitors to investigate if the lower lying times come from time spent grazing.

Cows spend around 54% of the night outside lying down, and this figure did not differ between the Pasture and the Sand phase. However, the total amount of time spent outdoors was lower during the Sand phase. We found no effect of weather conditions on any outcome measure, but we speculate that very wet conditions may have different effects on different types of outdoor surface depending upon cover, drainage, etc., and we encourage future work on this issue.

Cows spent less time perching during the day and over the 24-h period when they were provided outdoor access at night. This lower time spent perching may be beneficial to the cows' health, as perching is linked to lameness in dairy cattle (Bernardi et al., 2009; Fregonesi et al., 2009a; b). To our knowledge, this study is the first to show how outdoor access during the night affects the behaviour of cows during the day. Lobeck et al. (2011) showed that cows housed on a compost bedded pack had improved feet and leg health resulting in lower lameness rates compared to free-stall barns. Boyle et al. (2008b) reported that heifers housed on an outdoor wood-chip pad also showed more social, play, stretching and scratching a part of their body while standing with 1 leg raised, compared to heifers housed in a free-stall. In combination, these results indicate that cows can benefit from access to an outdoor bedded pack.

2.5 Conclusions

Cows exhibited a preference to spend much of the night outside when provided the opportunity under the relatively mild weather conditions encountered in the current study. The preference to be outdoors was greater for a large pasture than for a small outdoor sand pack.

Chapter 3: Dairy cow preference for access to an outdoor pack in summer and winter

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3.1 Introduction

There is a growing body of evidence that the public considers pasture access for dairy cows important (Schuppli et al., 2014; Hötzel et al., 2017), and that cows value access to pasture, especially at night (e.g. Legrand et al., 2009; Charlton et al., 2013; von Keyserlingk et al., 2017). Cows may use indoor housing as protection from rainfall (Legrand et al., 2009; Charlton et al., 2011a, 2013) and solar radiation (Schütz et al., 2009).

Pasture access is not always feasible, due to a lack of available pasture on some farms and environmental constraints, such as high rainfall that can render the soil soft and susceptible to damage from cows. An alternative to pasture is a deep-bedded outdoor pack. One advantage of an outdoor pack is that it can be used year-round, without concern about grass growth, soil compaction, etc. In addition, space requirements for an outdoor pack are generally lower than for pasture.

Providing cows free access to an outdoor pack could have beneficial effects on their health and behaviour. When indoor housed cows were given a choice between a free-stall pen and an indoor open pack, they spent more time lying and standing with 4 feet in the open pack (Fregonesi et al., 2009b). These authors also reported that cows spent less time standing on wet concrete and perching (i.e. standing with the 2 front feet in the lying-stall) when housed on an indoor open pack, behaviours that put cows at risk for lameness (Galindo and Broom, 2000). Another study confirmed that daily perching time decreased when cows were given access to an outdoor open sand pack or pasture (Smid et al., 2018). One concern with providing pasture access is that this will result in decreased feed intake (Schuppli et al., 2014). One previous study (Legrand et al., 2009), found that cows decreased their TMR intake (by 2.9 kg DM/d) when given access to pasture, but another study (Chapinal et al., 2010) found no reduction in TMR intake when cows were housed on pasture at night. A study by Motupalli et al. (2014) found a higher milk production in cows that had free access to pasture, compared to cows housed continuously indoors.

To date, most work on preference of cows to access the outdoors has been conducted under summer conditions (when pasture is most commonly offered), so the aim of our first experiment was to investigate dairy cow preference for access to an outdoor pack versus remaining inside a free-stall barn in the summer. However, one advantage of an outdoor pack is that access can be offered year-round, so the aim of the second experiment was to also investigate preference in the winter. By providing cows access to an outdoor area that did not contain grass, this study also provides insight into preference for outdoor access independent of grazing. We hypothesised that cows would prefer to spend time outside at night in the summer, and that this preference for nighttime access would be absent during the winter. In addition, we hypothesised that cows would spend less time outside when the weather was hot or rainy (in the summer) and rainy, snowy or windy (in the winter). We also hypothesised that daily feeding and lying time would not change but that cows would spend less time perching when given access to an outdoor pack regardless of season.

3.2 Materials and methods

3.2.1 Cows and treatment

The summer experiment took place from June to August 2016 and the winter experiment from October 2016 to February 2017. Both experiments were carried out at the Dairy Education and Research Centre (Agassiz, BC, Canada) of the University of British Columbia. The University of British Columbia Animal Care Committee approved both experiments and all procedures (Protocol A15 - 0082).

For the 2 experiments, a total of 96 and 108 pregnant, late lactation (> 100 DIM) Holstein cows were selected from the herd and assigned to 8 (Summer experiment) and 9 (Winter experiment) groups (i.e. 12 animals/group) respectively. Cows were randomly assigned to group, blocking for parity, with some balancing to achieve similar DIM, 305-d projected milk production and experience with an outdoor pack. In summer and winter experiments, parity averaged (mean \pm SD): 2.3 \pm 0.1 and 2.5 \pm 0.3; DIM: 240 \pm 17 and 232 \pm 31; projected 305-d milk production: $11,310 \pm 159$ kg and $10,958 \pm 466$ kg and previous experience with an outdoor pack in number of experienced animals was 2.5 ± 1.2 and 6.1 ± 1.5 respectively. The majority of the animals had spent time on pasture as growing heifers, and some had also been kept on pasture during dry periods. Two experienced observers assessed the body condition using a 5-point scale (1 = thin, 5)= obese) with quarter point increments following Edmonson et al. (1989), and the gait using a 5point scale (1 = healthy, 5 = severely lame; Flower and Weary (2006) of each cow prior to group formation. BCS averaged 3.3 ± 0.1 and 3.4 ± 0.3 , and gait score 1.9 ± 0.2 and 2.0 ± 0.2 , for the summer and winter experiment, respectively. Thin cows (i.e. BCS < 2.5) and severely lame cows (gait score 4 and 5) were not included in the study.

Groups were assigned to 1 of 2 experimental pens for 14 d. Each pen had access to a separate outdoor pack (see Figure 2.1 for the schematic of the experimental setup). Groups were given 5 d to stabilize before being provided access to the outdoor pack. Thereafter, starting after the animals came back from morning milking, groups were housed indoors for another 2 d and baseline observations of feeding, perching and lying behaviour were made (Baseline phase). Groups were then given access to the outdoor pack during a 2-d habituation period. To ensure that cows were familiar with the outdoor pack they were moved outside at 1030 h, 1400 h, 2000 h, 2200 h and 0430 h, if not already outdoors. After this habituation period, starting when cows returned to their pen after morning milking, cows were given free continuous access to the outdoor pack for 5 d (Choice phase). Two groups were tested at one time but were staggered in time such that the second group started when the Baseline phase of the first group had finished. Thus, the 2 groups did not have access to the outdoor packs at the same time.

3.2.2 Housing, management and diet

3.2.2.1 Indoor area

The two indoor pens were located in a mechanically ventilated (72" Artex Storm Fan, Artex Barn Solutions, Abbotsford, BC) wooden frame free-stall barn (42 x 93 m) with a north-south orientation and curtained sidewalls. Each pen consisted of 12 lying stalls, configured in 3 rows of 4 stalls that were filled with approximately 40 cm of washed river sand. Stalls were divided by Dutch-style partitions (Y2K stall dividers, Artex Barn Solutions, Abbotsford, BC) spaced 1.2 m wide centre-to-centre with the neck rail placed 1.3 m above the stall surface and 1.4 m from the inside of the rear curb. The 0.2 m high brisket board was placed 1.8 m from the inside of the curb, which was 0.2 m high as measured from the alley floor. The concrete alleys were cleaned a minimum of 6 times daily with an automated scraper; cross-over alleys were manually cleaned twice a day. Each pen had a headlock feed barrier with 12 headlocks per pen, 60 cm wide centre-to-centre.

Cows were fed a TMR formulated following the National Research Council (NRC) guidelines (NRC., 2001) to meet or exceed the requirements of a 658 kg Holstein producing a daily 36 kg of milk. During the Summer experiment, the TMR consisted of 30 % grass silage, 49 % concentrate mash, 14 % corn silage, 6 % grass hay and 1 % wheat straw on a DM basis; during the Winter experiment, the TMR consisted of 34 % corn silage, 44 % concentrate mash, 7 % grass silage, 7 % grass hay, 6 % alfalfa hay and 2 % wheat straw on a DM basis. TMR was available *ad libitum* inside throughout both experiments. In both experiments, fresh feed was provided between 0615 and 0645 h and between 1615 and 1645 h and pushed up at approximately 1045, 1845 and 2230 h; feed leftovers were taken away at approximately 0530 h. Water was available via a self-filling water trough (1.97 m x 0.54 m) located on the cross-over alley.

During both experiments, animals were milked twice daily in a double-12 parallel milking parlour, between 0615 and 0645 h in the morning and between 1615 and 1645 h in the afternoon. Animals that were outside were moved from the outdoor pack to the parlour; animals that were inside were moved from their pen to the parlour.

3.2.2.2 Outdoor area

The two outdoor packs each measured 144 m^2 and had a gravel surface bedded with a base layer of approximately 20 cm of sand and a top layer of approximately 25 cm of wood-chips (Figure 3.1). The outdoor packs were lined with electric fencing. One self-filling water trough (1.25 m x 0.84 m) was provided in each outdoor pack. Outdoor paths leading to the packs were covered with rubber mats. The paths and the outdoor pack were cleaned daily every afternoon when the cows were in the milking parlour.



Figure 3.1 Close-up of wood-chips used for the outdoor open pack.

3.2.3 Behavioural measures

During Baseline and Choice phases cows were recorded by video. Three dome video cameras (Panasonic WV-CW504SP, Sentinel Ultra-zoom w/Pan 1070 outdoor video camera, Sandpiper Technologies Inc., Manteca, CA) were attached on the outdoor wall of the barn 6 m above ground. The middle camera provided an overview of the entrances of both outdoor packs and the other two cameras each provided an overview of a single pack. These cameras were placed 6.1 m left and 7.0 m right from the middle camera. In addition, a dome camera was attached 8 m above the indoor alleys that connected the outdoor areas with the experimental pens, to allow the

observation of cows in the indoor alleys. Above each experimental pen a dome video camera was positioned at a height of 8 m to provide an overview of the indoor lying area. Another video camera (Panasonic WVCP-470, Panasonic Corporation of North America, NJ, USA) was placed 6 m above the feed bunk of each experimental pen. All video recordings were stored using a GeoVision 1480 digital recorder (USA Vision Systems, Irvine, CA). Infrared lights (BR38 Red Incandescent Flood Light 100 W, Globe Electric company INC., Montréal, QC) were placed adjacent to all in-and outside cameras to facilitate the observation of the cows during the night. Each cow was hair dyed with a unique symbol on both of her sides and on her back to facilitate individual recognition.

Feeding and stall perching behaviour of the cows inside the free-stall barn was scored using 5-min scan sampling from video during both the Baseline and Choice phases. Feeding was defined as the cow having her head completely through the headlock. A cow was considered perching if she was standing with only her two front feet in the lying stall. In addition, during the Choice phase, the location of animals (i.e. in the pen, on the outdoor pack, in the indoor- or outdoor alley) was also scored using 5-min video scan sampling.

HOBO data loggers (HOBO Pendant G, Onset, Cape Cod, MA) (validated by: (Ledgerwood et al., 2010) were used to quantify lying times (UBC AWP, 2013). The data loggers, attached to one of the rear legs of the cow, were programmed to record the posture of the cow, i.e. lying or standing, in 1-min intervals. The loggers were attached during milking at least 1 d before the beginning of the Baseline phase and were removed after the experimental period.

3.2.4 Climatic measures

For each experimental day, hourly mean air temperature (°C) and wind speed (m/s) and the hourly maximum relative humidity (%) and total precipitation (mm) were recorded at a weather

station 0.4 km from the test barn. The temperature-humidity index (THI) was calculated as: THI = $(1.8T + 32) - [(0.55 - 0.0055 \text{ RH}) \times (1.8T - 26)]$ with T = air temperature (°C) and RH = relative humidity (%) (Ravagnolo et al., 2000). Daily temperature, wind speed and relative humidity averaged (mean ± SD; numbers are given for the Summer and Winter experiment, respectively): 18.4 ± 2.3 °C (range: 13.9 - 23.5 °C) and 5.1 ± 4.6 °C (range: -4.3 - 13.5 °C); 4.9 ± 1.3 m/s (range: 2.5 - 8.2 m/s) and 8.0 ± 6.3 m/s (range: 1.3 - 23.9 m/s); 72.8 ± 7.2 % (range: 57.1 - 88.4 %) and 78.9 ± 16.6 % (range: 26.7 - 94.5 %). Precipitation was recorded on 14 of the 36 experimental days during the summer experiment and on 24 of the 39 experimental days during the winter experiment. Precipitation averaged 2.7 ± 4.6 mm (range: 0.2 - 16.9 mm) during the summer experiment and 9.9 ± 10.6 mm (range: 0.3 - 37.4 mm) during the winter experiment, on days with precipitation. THI averaged 63.5 ± 3.2 (range: 57.0 - 70.2) during the summer experiment and 43.4 ± 6.7 (range: 30.8 - 56.4) during the winter experiment.

3.2.5 Statistical analyses

A total of 7 cows (4 from the summer and 3 from the winter experiment) became lame during the study and were excluded from the analyses. One cow (from the winter experiment) refused to go outdoors during the habituation phase and was excluded from the analyses. On 2 occasions (once during the summer and once during the winter), a non-experimental cow was inadvertently added to the experimental group (on the last day of the Choice phase in the summer experiment, and on the first day of the Choice phase in the winter experiment). For one group in the winter experiment, on one occasion (on the second day of the Choice phase), cows were mixed with cows of an adjacent pen. The data from these days were excluded from the analyses. An additional 3 experimental days were lost (1 d from 3 different groups) during the summer experiment; on 2 occasions due to problems with the gates that separated the outdoor from the

indoor area and on another occasion due to several cows being out of the pen for hoof trimming. Malfunction of a camera resulted in the loss of feeding and perching behaviour data for one group for 2 d during the summer experiment. Extreme weather (snow and wind) during the winter experiment resulted in the doors that provided access to the outdoor pack being closed for 4 d (1 d for one group; 3 days for the other group).

Data were summarized by group (i.e. each group represented the average of 12 cows) and phase and analysed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC) treating group as the experimental unit. All results are reported as a percentage of time available for observation (i.e. not including times when cows were away for milking, health checks, etc.). In addition, with regards to the analysis of time spent inside or on the outdoor pack, observations in which cows were in standing in the indoor (i.e. the alley connecting the free-stall to the outdoor area) or outdoor alley were also removed. All analyses were run separately for summer and winter experiments. Using PROC UNIVARIATE, the histograms and QQ-plots of all variables were visually assessed for normality. In addition, all model residuals were visually assessed. For the analysis of time spent inside and outside, the data were analysed over the full daily period as well as separately for the day (0600 - 2000 h) and night (2000 - 0600 h). Differences in time cows spent outside during the day and night were tested using a paired t-test, using the average time groups spent outside during the day and night over the complete Choice phase (i.e. 5 d). Two out of the 9 groups of the winter experiment were extreme outliers, spending much more time outside than the other groups. These two groups were not included in the statistical analysis, but the results are reported descriptively in the text.

The PROC MIXED procedure in SAS was used to test the differences in time spent in different behaviours (i.e. feeding, perching and lying) during the Baseline and Choice phases, with

phase included as a fixed effect and group as random effect. A Spearman correlation was used to investigate the correlation between the weather variables (i.e. air temperature, wind speed, relative humidity, precipitation and THI) by season. Some of the environmental measures (e.g. temperature and THI) were highly correlated (r > 0.7). In these cases, we kept in the analysis only the correlate for which we had the strongest prediction. This resulted in dropping temperature, relative humidity and wind speed for the summer experiment, and relative humidity and THI for the winter experiment. Data were summarized by group and day; the effects of the weather conditions on the preference for outdoor access was tested using a mixed model with day as a repeated measure and group as subject. Significance was declared for P < 0.05 and a trend for P < 0.10. All means and standard errors reported below are derived from model output.

3.3 Results

3.3.1 Time spent in the free-stall versus on the outdoor pack

Cows averaged (mean \pm SE; min - max) 25.3 \pm 4.3%; range: 8.0 – 44.5% of their time on the outdoor pack in the summer and 1.8 \pm 0.6%; range: 0.1 – 4.1 % in the winter (Figure 3.2). In the summer, cows spent more time outside at night (50.0 \pm 8.4%; range: 15.8 – 84.2%) than during the day (3.3 \pm 1.3%; range: 0.9 – 11.4%; t7 = -5.56, *P* < 0.001). Groups varied in time spent outside in the winter: 7 of the 9 groups spent little time outside during the day (1.7 \pm 0.7%; range: 0.0 – 5.1%) or night (2.1 \pm 1.0%; range: 0.0 – 7.1%; t6 = -0.41, *P* = 0.693); the two other groups spent much more time outside during the day (averaging 8.9% in one group and 23.4% in the other) and especially so at night (50.7% and 46.8% for the two groups).

Figure 3.2 Time cows spent outdoors on a wood-chip pack when given a free choice during summer and winter

Mean (\pm SE) percentage of time groups of lactating dairy cows (summer: n = 8 groups; winter n = 7 groups) spent on the outdoor pack when provided a free choice between a free-stall barn and an outdoor pack during the summer (A) and winter (B).



3.3.2 Feeding, perching and lying behaviour

Cows spent more time feeding during the Baseline versus Choice phase in the summer (F_{1,7} = 12.10, P < 0.02; Figure 3.3). During the winter, no difference in feeding time was found between the phases. Time spent perching was higher during the Baseline versus Choice phase in summer (F_{1,7} = 21.59; P < 0.01) and tended to be higher in the Baseline versus the Choice phase in the winter (F_{1,6} = 5.58, P = 0.056). Daily lying time did not differ between Baseline and Choice phases during either summer or winter. When on the outdoor pack, cows averaged 53.7 (± 5.6) % of the time lying down during the summer and 4.7 (± 2.5) % during the winter.

Figure 3.3 Time cows spent on various behaviours

Mean (\pm SE) time groups of lactating dairy cows (summer: n = 8 groups; winter n = 7 groups) spent feeding, perching and lying, expressed as a percentage of the time available for observation (i.e. when not away from the pen for milking, etc.) during the Baseline (grey bar) and Choice (white bar) phases in the summer (A) and winter (B). Significance is indicated by an asterisk (*), a trend is depicted by a plus-sign (+).



3.3.3 Environmental conditions

During summer nights, cows spent less time outside when it was raining; time outside decreased by $14 \pm 5.5\%$ of the time available during the night for every 1 mm increase in hourly rainfall (F_{1,26} = 6.49, *P* < 0.02). During summer days, precipitation tended to increase the time cows spent outside (F_{1,26} = 3.11, *P* = 0.090) by approximately the same amount (14.3 ± 8.1% / mm). During winter days (for the 7 groups with low time spent outside), precipitation (F_{1,19} = 4.51, *P* < 0.05; slope -1.2 ± 0.6% / mm) and wind speed (F_{1,19} = 4.96, *P* < 0.05; slope -0.1 ± 0.1%/m/s) decreased time spent outside. Of the 2 outlier groups that spent much more time outside, one was tested during the warmest period encountered during the winter experiment (averaging 7.4 ± 1.5 °C), and these 2 groups were the only ones tested in the winter that experienced none or very little precipitation and were only exposed to low wind speeds.

3.4 Discussion

Cows spent about 25% of their time outside during summer; this value is lower than that reported in previous studies for pasture access (Legrand et al., 2009; Charlton et al., 2011b, 2013), suggesting that the outdoor pack used in this study was less attractive than pasture. This conclusion is consistent with previous work showing a preference for pasture when cows were provided a choice between pasture and an outdoor sand pack (Smid et al., 2018). Pasture allows cows to engage in grazing behaviour, possibly a rewarding activity for cows (Charlton and Rutter, 2017), which may explain this preference. In addition, the space available on the outdoor pack (i.e. 12 m2 per cow) was much smaller than the space on the pasture provided in previous studies (Legrand et al., 2009; Charlton et al., 2011b, 2013). Future work should investigate how space allowances affect preference for outdoor access.

In summer, cows spent less time outside during the day than during the night, likely to avoid solar radiation (Schütz et al., 2009). During summer days with precipitation cows tended to spend more time outside in our study, potentially because of the cooling effect of precipitation and the reduced solar radiation. Cows spent less time outside during summer nights and winter days with precipitation. This result is in line with other studies (Legrand et al., 2009; Charlton et al., 2011a, 2013). THI (summer) and air temperature (winter) did not affect the amount of time cows spent outside. The lack of an effect may be due to air temperatures that rarely exceeded the lower or upper critical temperature (LCT and UCT, respectively) of cattle. Chase (2011) estimated the LCT of lactating dairy cows to vary between -35 °C and -14 °C. The LCT is dependent on climatic factors (such as wind speed and precipitation) and individual characteristics (such as hair length and type and production level; Boyle et al., 2008). Berman et al. (1985) estimated the UCT of dairy cattle to be around 25 °C; air temperatures above this threshold cause an increase in body temperature that reduces milk production and induces heat stress (Kadzere et al., 2002). Daytime temperatures during the summer occasionally exceeded the UCT, but on average temperatures were much lower. Despite temperatures well above the LCT, cows spent little time outside during the winter. It is possible that the combination of various weather conditions (i.e. strong wind in combination with lower temperatures and the presence of snow on the outdoor pack) was aversive to the cows. The 2 groups that spent much more time outside during the winter experienced none or very little precipitation and low wind speeds. We used a within-group comparison in the current study; this design was likely helpful in accounting for this substantial variation among groups.

In the summer, but not during the winter, time spent feeding inside the barn was higher during the Baseline phase compared to the Choice phase, but the difference was small (1.3%). Legrand et al. (2009) noted a 60 min decline in feeding time at the feed bunk when cows were

given free access to pasture day and night (except for milking times) as compared to being housed fulltime indoors. That the cows in the current study did not have access to pasture and, hence had nothing to eat outdoors, may explain the lack of difference in feeding time.

Perching was reduced when cows were given access to the outdoor pack during the summer and tended to be lower in winter. Decreases in perching time when cows are allowed outdoor access are in line with the results of Smid et al. (2018). Bernardi et al. (2009) found a reduction of 16 min/d in time spent perching and a 26 min/d increase in time spent standing fully in the freestall when cows were provided access to less restrictive stalls; access to a less restrictive standing environment also improved gait scores in cows. As the outdoor pack provided cows with a less restrictive standing environment, they may have preferred to stand outside on the pack decreasing their perching times indoors.

When on the outdoor pack, cows spent about half of the time lying down in the summer. This result is in line with previous work that showed that cows spent approximately 54% of their time outside lying down (Smid et al., 2018). As suggested by Legrand et al. (2009), the lower lying times often observed when cows are kept on pasture versus free-stall housing (e.g. Hernandez-Mendo et al., 2007) may be due to time spent grazing. In addition, both studies suggested that cows might find pasture more comfortable for standing than the concrete flooring found indoors. Indeed, if flooring inside the barn is made more comfortable (Fregonesi et al., 2004; Tucker et al., 2006a; Boyle et al., 2007), standing times increase. During the winter experiment, cows spent around 5% of the time outside lying down. The observation that cows avoid lying down on bedding with high moisture levels (Reich et al., 2010), likely explains this result.

There are a number of potential limitations to the current study. One is that options tested (i.e. the free-stall barn and the outdoor pack) differed in many ways, so it is difficult to disentangle

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which factors were important to cows (e.g. TMR was only available indoors). A series of experiments where factors are varied individually (i.e. providing cows feed indoors and outdoors, providing cows access to a concrete area outside, etc.) could determine which aspects of the outdoors are most important.

A second limitation relates to the effect of experience. Previous experience with the available options can affect how animals respond when subjected to a preference test (Dawkins, 1977). In the current study animals were given 2 d to habituate to the outdoor pack and this period may not have been sufficient. Tucker et al. (2003) found that animals required long habituation periods (i.e. several weeks to months) to overcome preferences for various types of bedding. Future work should investigate whether longer habituation periods, including having experience as a growing heifer, affects preference for various types of outdoor access. More cows in the winter experiment had experience with the outdoor pack than cows used in the summer experiment; given that the winter cows used the pack less than the summer cows it would seem that the effects of experience were not so large as to overwhelm the effect of season.

A third limitation is the use of a 5-d test period to investigate preference for outdoor access. Preferences may change over time (Fraser and Matthews, 1997) and we encourage future research to investigate the longer-term measures of preference, and how access to the preferred environments may affect other aspects of welfare including health aspects such as mastitis and lameness.

Lastly, a preference test gives insight into an animals' preference for a specific resource but does not tell us what the strength of that preference is. When an animal is denied access to a resource for which its preference is strong, welfare is likely more affected than when this preference is weak (Fraser and Matthews, 1997). A motivation experiment can give more insight

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into the strength of the preference of animals for various resources. A recent study that trained dairy cows to push a weighted gate showed that cows were willing to work as hard to access pasture as they were to access fresh TMR, suggesting that access to pasture is highly valued by dairy cows (von Keyserlingk et al., 2017). We encourage future work to investigate the motivation of dairy cows to gain access to various outdoor areas.

3.5 Conclusions

During the summer, cows showed a partial preference for the outdoor pack and accessed the pack especially during the night. In the winter, cows showed little interest in accessing the outdoor pack. Access to the outdoor pack decreased perching time compared to when cows were housed indoors, with the effect being the strongest during the summer.

Chapter 4: Effect of outdoor open pack space allowance on the behaviour of free-stall housed dairy cows

A version of this chapter has been submitted for publication: A.M.C. Smid, D.M. Weary, and M.A.G. von Keyserlingk. Effect of space allowance on an outdoor open pack on the behaviour of free-stall housed dairy cows.

4.1 Introduction

Access to pasture is viewed as important for dairy cows in many countries (e.g. The Netherlands: Boogaard et al., 2008; US and Canada: Schuppli et al., 2014; Brazil: Hötzel et al., 2017), but is not always perceived as feasible by dairy farmers. Farmers face a range of practical constraints, including the lack of available pasture, particularly as farm size increases (Robbins et al., 2016). In addition, pasture access may not be feasible during parts of the year, for example, when the soil is wet and subject to damage from cow traffic. One alternative to pasture is a bedded outdoor area (i.e. Smid et al., 2018) where cows can move freely but that requires less space than pasture and can be used year-round.

Several studies have investigated the effect of space allowance on the behaviour of dairy cows. For example, Schütz et al. (2015) reported that a minimum of 6.0 m₂ of space allowance per cow was needed on a rubber pad during an 18 h stand-off period (when cows are kept off pasture in wet weather to prevent damage to grass) to maintain daily lying times similar to those observed when cows were housed on pasture. When cows were provided less space (3 or 4.5 m₂ of rubber mat per cow), the reduction in lying times and lying bouts was thought to be due to increased agonistic behaviour. Nielsen et al. (1997) found that heifers given 1.8 m₂/animal of lying space in

an indoor straw pack exhibited more agonistic interactions, including displacements from lying position, than when given 2.7 or 3.6 m² per animal. However, these studies all investigated the effect of space allowance in areas where cows had no other option. To our knowledge, no study has investigated how space allowance on an outdoor open pack affects cow behaviour and cow preference to be outdoors, when cows are given a free choice to access to a secondary area (in addition to the free-stall).

The aims of this study were to investigate how space allowance of an outdoor open pack affects: 1) the preference of free-stall housed cows to be outdoors; 2) the number of agonistic interactions on the outdoor pack, and 3) the proportion of outdoor time that cows spent lying. We hypothesised that at higher space allowances cows would spend more time outside, especially during the night when outdoor access is most popular. We also hypothesised that at higher space allowances cows would spend more time lying down outside and would engage in fewer agonistic interactions.

4.2 Materials and methods

4.2.1 Cows and treatment

This experiment took place between May and July 2017 at the Dairy Education and Research Centre (Agassiz, BC, Canada) of the University of British Columbia. The University of British Columbia Animal Care Committee approved the experiment and all procedures (Protocol A15 - 0082).

A total of 72 pregnant, lactating Holstein cows were randomly selected from the herd and assigned to 3 groups (24 animals/group). Groups were balanced (mean \pm SD) for parity (3.0 \pm 0.1), DIM (217 \pm 8), projected 305-d milk production (11604 \pm 263 kg), BCS (3.4 \pm 0.0) (scored on a
5-point scale, 1 = thin, 5 = obese, with quarter point increments following Edmonson et al. (1989), gait score (1.9 \pm 0.0) (5-point scale, 1 = healthy, 5 = severely lame, following Flower and Weary, 2006) as well as for experience with an outdoor pack (13-15 experienced cows per group). Cows that were in early lactation (< 100 DIM), thin (BCS \leq 2), or severely lame (gait score \geq 4) were not enrolled.

Animals were enrolled in the study for 21 d. At formation of each group (d 1), animals were given 3 d to allow for the social dynamics of the group to stabilize (see von Keyserlingk et al., 2008), followed by a 5 d habituation phase in which animals were given access to the outdoor pack with a space allowance of 16 m² per cow. To ensure that cows were familiar with the outdoor pack, they were moved outside (if not already outdoors) during the first 2 habituation days at 1030 h, 1400 h, 2000 h, 2300 h and 0600 h and during the last 3 d at 1100 h and 2300 h. After the habituation period, when cows returned to their pen after morning milking, the treatments commenced.

Thirteen different space allowances in the outdoor pack were tested, ranging from 4 - 16 m² per cow in 1 m² increments. Space allowance was applied randomly each day, without replacement, at approximately 0715 h while the cows were being milked. In addition, the indoor feeding area was blocked with a chain and all 24 cows were taken to the outdoor pack once they had returned from milking together. They were then held on the pack for 5 min before being moved indoors and allowed free access to the indoor feeding and lying areas.

Animals were milked twice daily in a double-12 parallel milking parlour, between 0715 h and 745 h in the morning and between 1700 and 1730 h in the afternoon. Cows were out of their pen for milking for approximately 45 min in the morning and 45 min in the afternoon.

4.2.2 Housing, management and diet

4.2.2.1 Indoor area

The indoor free-stall pen was located in a mechanically ventilated (72" Artex Storm Fan, Artex Barn Solutions, Abbotsford, BC) wooden frame free-stall barn (42 x 93 m) with a northsouth orientation and curtained sidewalls. The experimental pen consisted of 24 lying stalls, configured in 3 rows of 8 stalls, filled with \pm 40 cm of washed river sand, replenished biweekly. Stalls were raked twice daily, when cows were gone for milking; stalls were divided using Dutchstyle partitions (Y2K stall dividers, Artex Barn Solutions, Abbotsford, BC) spaced 1.2 m centreto-centre with the neck rail placed 1.4 m above the stall surface and 1.7 m from the inside of the rear curb. The 0.15 m high brisket board was positioned 1.8 m from the inside of the rear curb, which was 0.2 m in height as measured from the alley floor. The concrete alleys were cleaned 6 times daily with an automated scraper; cross-over alleys were manually cleaned twice a day when cows were away from the pen during milking. The pen had a post-and-rail feed barrier (see Huzzey et al., 2006) allowing 53 cm of feed bunk space per cow.

Cows were fed a TMR formulated following the National Research Council (NRC) guidelines (NRC, 2001) to meet or exceed the requirements of a 658 kg Holstein producing 36 kg of milk per day; the TMR consisted of 34 % corn silage, 44 % concentrate mash, 6.5 % grass silage and 7 % grass hay, 6.5% alfalfa hay and 2% wheat straw on a DM basis. TMR was available *ad libitum* inside during the complete experimental period. Fresh feed was provided between 0630 and 0700 h in the morning and between 1530 and 1600 h in the afternoon. Feed was pushed up at approximately 1045, 1845 and 2230 h and orts were taken away at approximately 0515 h. Animals had *ad libitum* access to fresh water, provided in one self-filling water trough located on the cross-over alley.

4.2.2.2 Outdoor area

The outdoor open pack (Figure 4.1) was 384 m₂ (i.e. 12 x 32 m) with a gravel base covered with a base layer of approximately 20 cm of washed river sand and topped with approximately 20 cm of wood-chips (a by-product of the forestry industry). Space allowance was varied by adjusting movable electric fencing (see Figure 4.1). The path connecting the pen and the outdoor pack were cleaned every morning and afternoon while the cows were being milked.

Figure 4.1 Schematic of experimental areas

Overview of the experimental areas used to test the preferences of lactating dairy cows to be in the free-stall barn or on the outdoor pack when provided various space allowances outside.



4.2.3 Behavioural measures

Three dome video cameras (Panasonic WV-CW504SP, Sentinel Ultra-zoom w/Pan 1070 outdoor video camera, Sandpiper Technologies Inc., Manteca, CA) were attached at a height of 6 m on the outdoor wall of the barn that collectively provided an overview of the entire outdoor pack. Above the experimental pen, two dome video cameras were positioned 8 m above the pen to provide an overview of the indoor lying area. Two additional video cameras (Panasonic WVCP-470, Panasonic Corporation of North America, NJ, USA) were placed 6 m above the feed bunk of the experimental pen to provide an overview of the feeding area. All video recordings were stored using a GeoVision 1480 digital recorder (USA Vision Systems, Irvine, CA). Infrared lights (BR38 Red Incandescent Flood Light 100 W, Globe Electric company INC., Montréal, Canada) were placed adjacent to each camera to facilitate the observation of the cows at night. Each individual cow was marked with hair dye to facilitate individual recognition.

The location of animals (i.e. in the free-stall pen or on the outdoor pack), and displacements from a lying position on the outdoor pack, were scored using continuous video observations. A displacement was defined by any movement or physical contact of the 'actor' cow, that was standing idle or walking within a body length distance from the lying reactor cow, towards a 'reactor' cow resulting in the 'reactor' cow transitioning to a standing position (i.e. 4 feet on the ground) within 15 s. Inter-observer reliability for this measure was $R_2 = 0.96$.

Lying times and the number of lying bouts were quantified with HOBO data loggers (HOBO Pendant G, Onset, Cape Cod, MA; UBC AWP, 2013) programmed to record cow posture (i.e. lying or standing) in 1-min intervals (validated by: Ledgerwood et al., 2010). The logger was attached to one of the cows' rear legs on the last day of the habituation period.

4.2.4 Climatic measures

Average hourly air temperature (°C), wind speed (m/s), the hourly maximum relative humidity (%), and rainfall (mm) were recorded at the Environment Canada weather station in Agassiz, B.C. located ± 400 m from the UBC Dairy Education and Research Centre. Temperature-humidity index was calculated as: THI = $(1.8T + 32) - [(0.55 - 0.0055 \text{ RH}) \times (1.8T - 26)]$ with T = air temperature (°C) and RH = relative humidity (%) (Ravagnolo et al., 2000). Daily temperature, wind speed and relative humidity averaged (mean \pm SD): 17.2 \pm 2.9 °C (range: 11.6 – 22.2 °C); 5.8 \pm 2.0 m/s (range: 3.5 – 14.6 m/s) and 67.5 \pm 10.1 % (range: 48.5 – 87.8 %). THI averaged 61.5 \pm 3.7 (range: 53.4 – 67.9). Precipitation was recorded on 6 out of 33 complete experimental (i.e. 24 h) days that were included in the analysis (see next section) and averaged 6.2 \pm 7.3 mm (range: 0.2 –19.8 mm).

4.2.5 Statistical analyses

One complete day (i.e. day and night period) (6 m₂ space allowance) from group 1 and one complete day (7 m₂ space allowance) from group 3 were excluded due to one cow in the group being in heat. Two nights (space allowances: 5 and 9 m₂, group 2 and 3, respectively) were excluded due to technical errors and 2 days (space allowances: 5 and 16 m₂, group 1 and 3, respectively) were excluded due to the delivery of new pole peelings in the pack on one day and sand bedding to the free-stall area on another day. One cow was diagnosed with severe mastitis on the 5th experimental day and moved to a hospital pen and not included in the analyses. A total of 7 cows were excluded from the analysis of lying behaviour, due to technical issues with the HOBO data loggers.

Data were summarized by group (i.e. each group represented the average of all cows in a group) and treatment, treating group as the experimental unit and analysed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC). Data were analysed over the full 24 h, as well as separately by day (i.e. time between morning and afternoon milking) and night (i.e. time between afternoon and morning milking). Model residuals were visually assessed for normality; 2 outliers were identified and deleted from all analyses: during 2 days of group 1, cows spent \pm 2 h outside during the day, which is much higher than the average time cows spent outside during the day. Both days were testing high space allowances (14 and 15 m₂/cow), had lower than average THI values and on one of these days, precipitation was recorded. A mixed model was used to test the effect of outdoor space allowance on time spent outside, frequency of visits to the pack as well as on various behaviours (i.e. the number of displacements on the outdoor pack, lying time outside, and daily lying time). With regard to lying time outside, days during which cows did not go outside were not included in the analysis. Space allowance and its quadratic term were included as fixed effects. A random intercept with group as subject was included, and an autoregressive type 1 covariance structure was specified. The correlation between several environmental conditions (i.e. air temperature, relative humidity, precipitation and THI) was investigated using a Spearman correlation. Air temperature, relative humidity and THI were highly correlated (r > 0.7). As THI is a composite measure of air temperature and relative humidity, we retained THI in the analysis and dropped the air temperature and relative humidity. As precipitation was recorded on only 6 out of 33 experimental days, we did not include rainfall in our models. In all models, THI as well as its quadratic term and order of testing the different space allowances were included as covariates. Significance was declared at P < 0.05, and a trend at P < 0.10.

4.3 Results

4.3.1 Time spent outdoors and visits to the outdoor pack during the day and night

Over the 24 h, cows spent more time on the outdoor pack with increasing space allowance $(F_{1,23} = 6.27, P = 0.0198, \text{ slope: } 0.5 \pm 0.2)$, and tended to do so in a diminishing manner $(F_{1,23} = 3.59, P = 0.0706, \text{ slope: } -0.02 \pm 0.01)$. However, this was almost exclusively driven by the increased time cows spend outdoors during the night time hours. Hence, we did not analyse the 24 h period any further, but rather analysed the day and night period separately.

During the day, space allowance did not affect the time cows spent outside (F_{1,25} = 1.08, P = 0.3095) (Figure 4.2A). However, increasing THI decreased the time cows spent outside (F_{1,25} = 22.42, P < 0.001, slope: -0.9 ± 0.2) with a quadratic term indicating that this tended to do so in a decelerating way (F_{1,25} = 21.34, P < 0.001, slope: 0.01 ± 0.00).

At night cows spent more time outside with increasing space allowance (F_{1,27} = 7.68, P = 0.0100, slope: 0.5 ± 0.2), with the quadratic term reflecting a diminishing effect (F_{1,27} = 5.32, P = 0.0290, slope: -0.02 ± 0.01) (Figure 4.2B). In addition, increasing THI decreased the time cows spent outside during the night (F_{1,27} = 6.34, P = 0.0180, slope: -2.7 ± 1.1), in an increasing manner (F_{1,27} = 6.43, P = 0.0173, slope: 0.02 ± 0.01).

The frequency of visits to the outdoor pack did not differ with space allowance during the day (F_{1,25} = 1.25, P = 0.2734) or night (F_{1,27} = 0.19 P = 0.6645). However, during the day, increasing THI decreased the number of visits to the outdoor pack (F_{1,25} = 12.20, P = 0.0018, slope: -1.0 ± 0.3), in an increasing manner (F_{1,25} = 11.90, P = 0.0020, slope: 0.01 ± 0.00).

Figure 4.2 Time lactating dairy cows spent on the outdoor pack with various outdoor space allowances during the day and night.

Average time lactating dairy cows (n = 3 groups of 24 cows; group 1 indicated by round dots; group 2 indicated by triangle dots; group 3 indicated by squared dots) spent on the outdoor pack with various space allowances during the day (A) (i.e. between morning and afternoon milking) and during the night (B) (i.e. between afternoon and morning milking). Over all space allowances, time spent outside during the day was (mean \pm SD): 0.3 ± 0.3 h; 0.1 ± 0.2 h and 0.1 ± 0.1 h for group 1, 2 and 3 respectively; time spent outside during the night was (mean \pm SD): 6.2 ± 0.9 h; 2.5 ± 0.9 h and 4.9 ± 0.9 h for group 1, 2 and 3 respectively.



4.3.2 Agonistic interactions and lying time

As cows generally spent little time outside during the day, the effect of outdoor space allowance on agonistic interactions and lying time on the pack was investigated for the night time only. Outdoor space allowance did not affect the number of agonistic interactions on the outdoor pack ($F_{1,27} = 1.26$, P = 0.2708). In addition, the proportion of outdoor time that cows spent lying was not affected by space allowance ($F_{1,27} = 1.82$, P = 0.1886). However, higher THI decreased the time cows spent lying on the outdoor pack ($F_{1,27} = 5.33$, P = 0.0288, slope: -0.2 ± 0.1), in an increasing manner ($F_{1,27} = 5.65$, P = 0.0248, slope: 0.0 ± 0.0). Total daily lying time (i.e. inside the free-stall barn and on the outdoor pack) was not affected by outdoor space allowance ($F_{1,23} = 0.01$, P = 0.9231), but increased with higher THI ($F_{1,23} = 10.73$, P = 0.0033, slope: 2.0 ± 0.6), in a diminishing manner ($F_{1,23} = 11.80$, P = 0.0023, slope: -0.02 ± 0.00).

4.4 Discussion

Cows spent more time outside with increasing outdoor space allowance over the 24 h period. This effect was driven by cows spending more time outside during the night than during the day: cows spent more time outside with increasing outdoor space allowance during the night, but this effect was absent during the day. Dairy cow preference for outdoor access during the night has been found in many other studies (e.g. pasture: Legrand et al., 2009; Charlton et al., 2011, 2013; von Keyserlingk et al., 2017; wood-chip pack: Smid et al., 2019). Increasing outdoor THI values decreased the time cows spent outside, the availability of mechanical ventilation inside the barn may at least partially explain this result. Other studies conducted in the same season and geographic region found a decrease in the amount of time cows spent on pasture with increasing outdoor THI during the day (Legrand et al., 2009; Falk et al., 2012). That THI values are generally

higher during the day than during the night, and given that cows avoid solar radiation (Schütz et al., 2009), collectively explains the strong preference of cows for night-time as opposed to day-time outdoor access.

Outdoor space allowance did not affect the frequency of visits to the outdoor pack during the day or night time. During the day, increasing outdoor THI decreased the frequency of visits to the outdoor pack. The decreased frequency of visits is likely explained by the fact that cows spent less time outside with increasing THI. A decreasing effect of outdoor THI on time spent outside was found by Legrand et al. (2009). However, Charlton et al. (2011b) observed the opposite effect. It is possible that other weather influences, such as the amount of solar radiation, account for these difference results. In addition, the THI range as reported by Charlton et al. (2011b) was lower than the range reported by Legrand et al. (2009). In our study, given that cows spent less time outside during the day than during the night, the effect of THI on frequency of visits to the outdoor pack may have been stronger during the day than during the night.

Based in part on the work of Schütz et al. (2015), the minimum space allowance on the outdoor pack was set at 4 m² per cow. The maximum space allowance on the outdoor pack was set at 16 m² per cow; equivalent to 50% more space per cow than the current recommendation set by the Canadian Code of Practice (NFACC, 2009) for open packs. During the night, when cows spent more time outdoors, outdoor space allowance had a positive effect on the time outdoors, with a quadratic term indicating a diminishing trend. This result suggests that increasing outdoor space allowance affects cow behaviour, but with diminishing returns. Hence, the range of space allowances as chosen for this study seems to have provided cows with enough variation in outdoor space allowance to reliably assess the effect on usage.

In contrast to our hypothesis, we found no effect of outdoor space allowance on the number of agonistic interactions between cows on the outdoor pack. In general, lower space allowances are related to a higher level of agonistic interactions between cattle (e.g. Nielsen et al., 1997; Talebi et al., 2014; Tresoldi et al., 2015). However, the cows in the current study always had free access to the free-stall barn. Thus, individual cows could avoid these interactions by simply moving indoors. The type of agonistic interactions may also have played a role. Schütz et al. (2015) found a decrease in the number of agonistic interactions between cows with increasing space allowance when confined to an outdoor space covered with a rubber mat. This effect was driven by interactions between standing cows; displacements from a lying position were uncommon and only nonaggressive interactions towards lying cows were affected by space allowance; aggressive interactions towards lying animals were not affected. Given the challenge of discriminating between aggressive and non-aggressive interactions on the outdoor pack, our definition of a displacement involved any type (i.e. aggressive and non-aggressive) of interaction between cows that resulted in the reactor cow changing from a lying to a standing position. It is unknown if space allowance influenced other types of agonistic interactions than displacements from a lying position. Val-Laillet et al. (2008) showed that more than 85% of all agonistic interactions between cows housed in a stable group in the free-stall barn occurred at the feed bunk. Given that no feed was provided outdoors, this may also partially have accounted for the lack of an effect.

No difference in the proportion of time cows spent lying outside during the night was found over the different outdoor space allowances. When confined to an outdoor space covered by a rubber mat, cows spent more time lying with increasing space allowance (Schütz et al., 2015) and heifers provided 1.5m₂/animal spent less time lying than when provided 3m₂/animal in either a pen with slatted flooring or slatted flooring with a straw bedded pack (Hindhede et al., 1996). Fisher

et al. (1997) showed that beef heifers kept in slatted floor pens at 1.5 m₂/heifer had reduced lying times compared to when provided 2.0, 2.5 or 3.0 m2/heifer. However, Mogensen et al. (1997) found no difference in lying time between heifers housed in straw bedded packs when provided 1.5 or 3.0 m₂/heifer but did note increased lying synchrony at the higher space allowance. Higher stocking density also reduces lying time in cows kept in free-stalls (Fregonesi et al., 2007a; Telezhenko et al., 2012). Given that daily lying time did not change with varying outdoor space allowances, cows in the current study compensated by lying inside the free-stall barn. As lying is a highly motivated behaviour in cows (Munksgaard et al., 2005; Tucker et al., 2018), it is not surprising that cows maintained their lying time. Nielsen et al. (1997) found no effect of space allowance of an indoor open pack on lying time in heifers but found that heifers would only lie outside of the bedded lying area when given the lowest lying space of 1.8m₂/animal, again suggesting that cattle try to maintain their lying times. Given that the cows in the present study spent more time outdoors with higher space allowance on the outdoor pack during the night but did not increase the relative proportion of lying time with increasing space allowance, indicates that they spent more absolute time standing outside with increasing outdoor space allowance. As suggested by Fregonesi et al. (2004), cows may prefer to stand on softer, rather than hard surfaces. As only concrete was available inside the free-stall barn, cows may have preferred to stand on the outdoor pack, given the softer standing surface outside.

Increasing outside THI decreased the proportion of outdoor time that cows were lying during the night, likely because a standing posture may facilitate heat dissipation (Wang et al., 2018). Interestingly, increasing outdoor THI increased total daily lying time. As the cows spent more time inside with higher THI values, they may have elected to lie down rather than stand on the concrete floor indoors (Fregonesi et al., 2004). The use of mechanical ventilation inside the barn may have facilitated heat dissipation, and thereby minimized the effect of THI on lying time.

4.5 Conclusions

Free-stall housed cows spent more time outdoors with increasing space allowance on an outdoor open pack during the night, but not during the day. When tested during the night, outdoor space allowance did not affect the number of agonistic interactions between cows outside nor did it affect the proportion of outdoor time that cows spent lying.

Chapter 5: The effects of access to an outdoor open pack on the expression of oestrus behaviour in Holstein cows

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5.1 Introduction

The reproductive efficiency of dairy cows has declined, perhaps in part due to increased use of confinement indoor housing (Lucy, 2001), which can impair oestrus expression especially due to the use of concrete flooring (e.g. Britt et al., 1986; Vailes and Britt, 1990). Oestrus behaviours include primary and secondary oestrus signs. Standing mounts, when cows stand immobile to be mounted by others, is the primary sign of oestrus (Diskin and Sreenan, 2000; Forde et al., 2011; Sveberg et al., 2011). Secondary signs include various types of mounting behaviour and subtler behaviours such as chin resting (Sveberg et al., 2011). There is evidence that pasture access improves the expression and detection of oestrus (e.g. Palmer et al., 2010, 2012). Cows on pasture stand to be mounted more often than cows housed in a free-stall barn (Palmer et al., 2010, 2012), and the odds of successfully detecting a cow in oestrus based upon behavioural observations is 2.3 times higher when cows are on pasture compared to indoors (Cutullic et al., 2009).

The use of concrete flooring in barns may help account for the lower expression of oestrus behaviours. Concrete flooring can inhibit natural locomotor behaviour (Telezhenko and Bergsten, 2005), especially if flooring is wet and slippery (van der Tol et al., 2005), possibly making cows more hesitant to mount due to the risk of falling (Boyle et al., 2007). Palmer et al. (2012) found that cows in a free-stall barn had more slips and falls when attempting to mount other cows compared to cows housed on pasture; Boyle et al. (2008b) showed that heifers housed on an outdoor open wood-chip pack had fewer slips, trips and falls compared to heifers housed in a freestall barn. When cows were able to choose between mounting a cow on concrete or dirt, they were 3 times more likely to mount on dirt (Vailes and Britt, 1990).

Providing cows free access to an outdoor pack, with high traction footing, may improve oestrus expression. The aim of this study was to investigate if providing cows access to an outdoor open pack increased oestrus behaviour. We hypothesized that providing free-stall housed cows access to an outdoor bedded pack would increase the expression of oestrus behaviours.

5.2 Materials and methods

5.2.1 Cows and treatment

This experiment took place between July 2017 and February 2018 at the Dairy Education and Research Centre (Agassiz, BC, Canada) of The University of British Columbia. The University of British Columbia Animal Care Committee approved the experiments and all procedures (Protocols A15 - 0082 and A14 - 0290).

A total of 60 lactating Holstein cows were used as experimental animals. Cows were randomly assigned to 1 of 2 treatments at calving: OUTDOOR cows were housed in a free-stall pen and allowed free access to an outdoor wood-chip pack; INDOOR cows were kept in the same free-stall pen (hereafter referred to as the test pen) but did not have access to the outdoor pack. Cows were balanced (mean \pm SD) for parity (OUTDOOR: 2.7 \pm 1.6; INDOOR: 2.5 \pm 1.3), if assistance was required at calving (OUTDOOR: 5 cows; INDOOR: 4 cows) and if cows had previous experience with an outdoor open pack (OUTDOOR: 15; INDOOR: 13). In addition,

multiparous cows were balanced for previous 305-d mature equivalent (305ME) milk yield (OUTDOOR: $12,423 \pm 2222 \text{ kg}$; INDOOR: $12,723 \pm 2004 \text{ kg}$).

Experimental cows were kept in the test pen for 84 ± 1 d (range: 83 - 89 d) after which no data were collected.

5.2.2 Housing, management and diet

Animals were housed in a in a mechanically ventilated (72" Artex Storm Fan, Artex Barn Solutions, Abbotsford, BC) wood-frame free-stall barn (42 x 93 m; Figure 5.1) with a north-south orientation and curtained sidewalls. The test pen consisted of 36 lying stalls, configured in 3 rows of 8 stalls and 3 rows of 4 stalls. Stalls were filled with ± 40 cm of washed river sand, replenished every other week. Stalls were divided by Dutch-style partitions (Y2K stall dividers, Artex Barn Solutions, Abbotsford, BC) spaced 1.2 m wide centre-to-centre with the neck rail placed 1.4 m above the stall surface and 1.7 m from the inside of the rear curb. The 0.15 m high brisket board was placed 1.8 m from the inside of the rear curb, which was 0.2 m high as measured from the alley floor. The concrete alleys were cleaned a minimum of 6 times daily with an automated scraper; cross-over alleys were manually cleaned twice a day. In the test pen cows had access to fresh water from a self-filling water trough located at the cross-over alley.





An automated selection gate (Lely Grazeway[™], Maassluis, The Netherlands) allowed OUTDOOR cows free access to the open pack from the test pen. The outdoor open pack was 384 m² and consisted of approximately 20 cm of sand covered by approximately 25 cm of wood-chips. In addition, the pack contained 2 self-filling water troughs. Faeces were removed every morning and afternoon when the cows were away being milked.

Cows were fed a TMR formulated following the National Research Council (NRC) guidelines (NRC., 2001) to meet or exceed the requirements of a 658 kg Holstein producing a daily

39 kg of milk; the TMR consisted of 30.3 % corn silage, 42.3 % concentrate mash, 5.9 % grass haylage, 1.5% grass hay, 19.2 % alfalfa hay and 0.7% wheat straw on a DM basis was available *ad libitum* inside the barn. Fresh feed was provided at approximately 0700 and 1630 h. Feed was pushed up at approximately 1045, 1845 and 2230 h and orts were removed at approximately 0530 h.

Animals were milked twice daily in a double-12 parallel milking parlour, between 0700 and 0800 h in the morning and between 1630 and 1730 h in the afternoon. Given that this study was not powered to test the effect of access to an outdoor pack on milk production, we will not discuss milk production further. Animals that were outside were moved directly from the outdoor pack to the parlour.

5.2.3 Group formation and training

Initially, 36 non-experimental (filler), open, lactating cows were housed in the experimental pen. Eighteen of these cows were trained and allowed access to the outdoor pack and the other 18 were not trained and kept indoors. OUTDOOR cows (both filler and experimental cows) were trained to go through the GrazewayTM that allowed them to go outdoors. INDOOR cows (both filler and experimental cows) were not trained; these animals could also access the GrazewayTM but were redirected back to the indoor alley that led to the home pen (Figure 1). All animals were equipped with an SCR collar (Heatime, SCR Engineers, Netanya, Israel) that was recognized by the GrazewayTM to direct them relative to their assigned treatment.

Training of experimental and filler cows was done over several sessions. The first session consisted of encouraging cows to approach the Grazeway[™] and to go through it, with all gates (i.e. sorting gate, and front and back gate) open, to access the indoor alley where they were

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rewarded with grain, fresh TMR and alfalfa hay. Cows were allowed access to the food rewards for approximately 1-2 minutes, before being moved back to the pen through the one-way gate. The one-way gate was initially kept fully opened, then half closed, and then fully closed for the final training sessions. Position of the one-way gate was changed to a more closed position once all cows of the group went through the one-way gates without hesitation. Each training session included a total of 4-5 loops through the GrazewayTM; a maximum of two sessions occurred per day. When all cows met the learning criterion -i.e. went through the open GrazewayTM without hesitation - the GrazewayTM was set on operating mode (i.e. gates automatically opened and closed). At this point animals were directed to the outdoor pack after entering the Grazeway[™] where they could access their reward for approximately 3 min. Again, animals were required to complete 4-5 successful loops within one session. After the initial set of animals were trained, they were used to facilitate the training of naïve animals. Specifically, an experienced cow would enter the GrazewayTM after which the naïve cow would be encouraged to follow. Training was done in the home pen directly after the morning and afternoon milking using groups of 3-5 naïve cows that had all calved within 9 d. All experimental animals met the learning criterion (of going through the GrazewayTM without a trainer present) 6 ± 2.3 d (range: 1 - 10 d) after entering the experimental pen.

Once all filler cows were successfully trained and were using the gate independently of human presence, the experiment started. Each time a cow calved, she was included in the experimental pen immediately following the first milking after calving, replacing a filler cow. Once 60 experimental cows had been enrolled in the study, fresh filler cows were used to replace experimental cows that were > 84 DIM.

5.2.5 Behavioural measures

Cow behaviour was continuously monitored using video recordings. Three dome video cameras (Panasonic WV-CW504SP, Sentinel Ultra-zoom w/Pan 1070 outdoor video camera, Sandpiper Technologies Inc., Manteca, CA) were attached at the outdoor wall of the barn at a height of 6 m to provide an overview of the complete outdoor pack; 2 were positioned 8 m above the experimental pen to provide an overview of the indoor lying area and 2 were positioned above the alley to provide an overview of this area and the GrazewayTM. Three other video cameras (Panasonic WVCP-470, Panasonic Corporation of North America, NJ, USA) were placed 6 m above the feed bunk to provide an overview of the feed bunk and alley. All video recordings were stored using a GeoVision 1480 digital recorder (USA Vision Systems, Irvine, CA). Red lights (BR38 red incandescent flood light, 100 W; Globe Electric Co. Inc., Montréal, QC, Canada) were placed adjacent to each camera to facilitate observation of cows at night. Each cow was dyed with a unique symbol on her back and sides to facilitate individual identification. For the duration of all oestrus periods of each cow, primary and secondary oestrus behaviours (Table 5.1) were scored by continuous video observations. In addition, the location (i.e. in the pen, the indoor alley or in the outdoor pack) where each behaviour was performed was also scored. Inter-observer reliability for each individual behaviour was $R_2 > 0.89$; inter-observer reliability for scoring cow location was $R_2 = 1.0$.

Behaviour*	Definition
Standing to be mounted	The cow stands still whilst another cow mounts her from behind for at least 2 seconds1 (i.e. the front legs of the mounting cow will move in front of the cows' Pelvic bone). She does not try to move away but may move a few steps over the duration of the mount to
Mounting performed	balance the weight of the other animal. The cow raises her body above that of another cow and clasps with her front legs in front of the other cows' Pelvic bone. The cow that is being mounted may or may not walk away
Attempted mount performed	The cow tries to mount another cow but is unsuccessful (i.e. the cow does not clasp her front legs in front of the other cows' Pelvic bone) as the cow moves away forwards, sideways or backwards from her or because she is physically unable to.
Attempted mount received	The cow moves away forwards, sideways or backwards from another cow that is attempting to mount her or because the performing cow is physically unable to.
Disoriented mount performed	The cow raises her body above that of another cow and clasps with her front legs in any place other than the front of the other cows' Pelvic bone
Disoriented mount received	The cow is being mounted by another cow that clasps with her front legs in any place other than the front of the cows' Pelvic bone.

Table 5.1 Ethogram describing the oestrus behaviours observed

* For each behavioural event the location where this event took place (i.e. inside the pen or on the outdoor pack) was noted. Ethogram is partially based on Palmer et al. (2012). 1Following Sveberg et al. (2011)

Animals wore an AFI leg-mounted pedometer (AfiActII Pedometer Plus, Afimilk, Kibbutz Afikim, Israel), used to detect oestrus events. Following an alert from the AFI pedometer, cows were checked for the presence of a preovulatory follicle and the absence of a mature corpus luteum (CL) by rectal ultrasonography following milking as well as 48 h and 7 d thereafter to confirm ovulation. Both suspected oestrus and false alerts were scanned 7 d post-alert to confirm oestrus classification. Duration of each oestrus event was calculated as the number of hours the cow spent above the threshold set by the manufacturer.

Two experienced observers assessed BCS during each oestrus event. BCS was assessed using a 5-point scale (1 = thin, 5 = obese) with quarter point increments, following Edmonson et al. (1989). In addition, cows were gait scored at every oestrus event using a 5-point scale (1 = healthy, 5 = severely lame; following Flower and Weary, 2006).

5.2.6 Climatic measures

Air temperature and relative humidity inside the barn were recorded in 10-min intervals using a Hobo U23 Pro v2 Temperature/Relative Humidity Data Logger (Onset Computer Corp.) placed at a height of 3 m in the middle of the experimental pen. Temperature-humidity index was calculated as: THI = $(1.8T + 32) - [(0.55 - 0.0055 \text{ RH}) \times (1.8T - 26)]$ with T = air temperature (°C) and RH = relative humidity (%) (Ravagnolo et al., 2000) and averaged 54.4 ± 10.2.

5.2.7 Statistical analyses

Due to severe winter weather conditions, the experiment was paused from the 26th of December 2017 until the 22nd of January 2018. During the complete experimental period (i.e. excluding the winter break) a total of 123 true oestrus events were recorded. Thirteen of these events could not be included in the analysis: during 6 events (2 OUTDOOR and 4 INDOOR cows), the AFI monitoring system had technical issues, on another occasion, an INDOOR cow obtained outdoor access during her oestrus, and 2 oestrus events of 2 OUTDOOR cows each were deleted as these cows were cystic during these estruses. In another instance, an OUTDOOR cow obtained access to a non-experimental pen during her oestrus event and in 4 instances, an INDOOR cow went through the Grazeway[™] and ended up in the indoor alley for several hours. One cow (INDOOR) died due to a severe mastitis infection, approximately 2 months after calving, and was

not included in the analysis; she was replaced by a filler cow. Three OUTDOOR cows were excluded due to failure of SCR collars, causing them to be regularly sent indoors by the GrazewayTM.

Data were analysed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC), treating 'cow' as the experimental unit. Behaviours in the different sub-categories (Table 5.1) were generally observed too infrequent to be able to analyse these separately and hence, the total number of oestrus behaviours was calculated per oestrus event per cow and expressed as frequency observed per hour of oestrus (i.e. oestrus intensity). Model residuals of oestrus behaviour were visually assessed for normality, which was attained, and for outliers, which were not identified. A mixed model using Type-1 Sum of Squares was used to investigate the effect of treatment (i.e. INDOOR vs OUTDOOR) on oestrus intensity as well as on oestrus duration. In addition to treatment as fixed effect, the following covariates were also included in the model, in the following order: parity (i.e. primi- or multiparous), DIM, gait score, BCS, time of oestrus onset as indicated by the AFI system, indoor THI, total number of cows in heat, number of outdoor cows in heat. The latter 2 covariates indicate the number of cows (respectively the total number of INDOOR and OUTDOOR cows and the number of OUTDOOR cows only) that were in true heat at the same time of the cow of interest, with at least 1 h overlap of their estruses as indicated by the AFI monitoring system. In addition, the interaction between each covariate and treatment was also included. The model for each dependent variable was created using backwards stepwise elimination. Cow was included as repeated statement and an autoregressive Type 1 covariance structure was specified. Given the use of repeated measurements, all cows with only 1 oestrus event were excluded from this analysis.

The effect of location on oestrus expression within OUTDOOR cows was investigated using a similar model and approach, with location (instead of treatment) tested as a fixed effect and location within oestrus event specified in the repeated statement. Given the use of within-oestrus event variation for this analysis, cows with only 1 oestrus event were used in this analysis. For both models, trends (i.e. P < 0.1) were retained in the model. Significance was accepted at P < 0.05.

5.3 Results

5.3.1 General results

A total of 3 OUTDOOR and 6 INDOOR cows did not have any true oestrus event during the experimental period. Of the cows that had at least 1 true oestrus event, 4 OUTDOOR cows and 7 INDOOR cows did not show any of the scored oestrus behaviours during at least 1 event (i.e. silent heat); 2 of these INDOOR cows did not show any of the scored oestrus behaviours during 2 oestrus events. The positive predictive value, defined by Burnett et al. (2018) as 'the number of oestrus alerts that correctly identified a cow in oestrus divided by the total alerts on the monitor,' averaged (\pm SE) 0.79 \pm 0.06 for OUTDOOR cows and 0.81 \pm 0.05 for INDOOR cows.

5.3.2 Effect of treatment on oestrus behaviours

We found an interaction between indoor THI and treatment (F_{1,46} = 6.30, P = 0.016), with INDOOR cows having a lower oestrus intensity with increasing indoor THI, an effect absent for OUTDOOR cows (Figure 5.2). The number of cows in heat positively influenced oestrus intensity (F_{1,46} = 33.59, P < 0.001). Table 5.2 provides an overview of the expression of the different oestrus behaviours as performed by OUTDOOR and INDOOR cows separately. **Figure 5.2 The effect of indoor THI on oestrus intensity in OUTDOOR and INDOOR cows** Relationship between indoor THI and the number of hourly oestrus events expressed by OUTDOOR cows (red line) versus INDOOR cows (blue line), including 95% confidence limits.



Table 5.2 Oestrus intensity of INDOOR and OUTDOOR cows

Raw means $(\pm SE)$ of the number of oestrus behaviours performed per hour of true heat by OUTDOOR vs INDOOR cows. Numbers are for descriptive purposes only.

	Treatment		
	OUTDOOR	INDOOR	
Standing to be mounted	0.3 ± 0.1	0.4 ± 0.1	
Mount performed	0.5 ± 0.1	1.0 ± 0.3	
Attempted mount performed	0.3 ± 0.1	0.4 ± 0.1	
Attempted mount received	0.1 ± 0.0	0.7 ± 0.2	
Disoriented mount	0.2 ± 0.1	0.4 ± 0.2	
Disoriented mount received	0.2 ± 0.1	0.3 ± 0.1	

5.3.3 Effect of treatment on oestrus duration

OUTDOOR cows had a longer oestrus duration than did INDOOR cows ($F_{1,27} = 4.30$, P = 0.048; 12.7 ± 0.8 h versus 9.8 ± 0.9 h respectively). In addition, DIM ($F_{1,49} = 10.53$, P = 0.002) and the number of cows in heat ($F_{1,49} = 4.80$, P = 0.033) both increased oestrus duration.

5.3.4 Effect of location on oestrus behaviours

We found an interaction between indoor THI and location; OUTDOOR cows showed lower intensity estruses with increasing THI, but only for behaviours expressed indoors ($F_{1,47} = 4.34$, P = 0.043; Figure 5.3). In addition, the number of cows in heat positively influenced oestrus intensity ($F_{1,47} = 7.76$, P = 0.008). Table 5.3 provides an overview of the expression of the different oestrus behaviours by location separately.

Figure 5.3 The effect of indoor THI on oestrus intensity of OUTDOOR cows outdoors and in the free-stall barn

Relationship between indoor THI and the number of hourly oestrus events expressed by OUTDOOR cows in the outdoor open pack (red line) versus in the free-stall (blue line), including 95% confidence limits.



Table 5.3 Oestrus intensity of OUTDOOR cows outdoors and in the free-stall barn

Raw means $(\pm SE)$ of the number of oestrus behaviours performed per hour of true heat by OUTDOOR cows in the free-stall versus on the outdoor open pack. Numbers are for descriptive purposes only.

	Treatment		
	OUTDOORS	FREE-STALL	
Standing to be mounted	0.1 ± 0.0	0.3 ± 0.1	
Mount performed	0.5 ± 0.2	0.5 ± 0.1	
Attempted mount performed	0.1 ± 0.0	0.3 ± 0.1	
Attempted mount received	0.5 ± 0.1	0.2 ± 0.1	
Disoriented mount performed	0.2 ± 0.1	0.2 ± 0.1	
Disoriented mount received	0.1 ± 0.1	0.2 ± 0.1	

5.4 Discussion

An increase in indoor THI reduced oestrus intensity (i.e. the hourly number of oestrus behaviours expressed), with INDOOR cows showing a decreased oestrus intensity with increasing indoor THI. This result is consistent with previous work showing that a period of high environmental temperatures decreases oestrus intensity in cows (reviewed by: Orihuela, 2000). In the present study, OUTDOOR cows were able to access the outdoor pack, perhaps providing a cooler environment especially during the night. Indeed, the number of oestrus behaviours that OUTDOOR cows performed inside the free-stall barn decreased with increasing THI, but no such decline was observed on the outdoor pack. Other studies have shown a higher expression of oestrus behaviours on pasture compared to a free-stall barn (Palmer et al., 2010, 2012) and on outdoor dirt lots compared to an outdoor concrete floor (e.g. Britt et al., 1986; Vailes and Britt, 1990), but these previous studies did not allow cows to choose between an indoor and an outdoor environment. The current study is the first to show that the benefits of outdoor access are greater at higher environmental temperatures.

Another important factor influencing oestrus intensity was the number of cows in heat, a finding also reported by Hurnik et al. (1975) and by Van Vliet and Van Eerdenburg. (1996). Animal in oestrus are more receptive to engage in oestrus behaviours (Sveberg et al., 2011), explaining this phenomenon.

When specifically looking at oestrus duration, differences in environmental THI may also, at least in part, explain the longer oestrus duration of OUTDOOR compared to INDOOR cows, as a period of high environmental temperatures also decreases oestrus duration in cows (reviewed by: Orihuela, 2000). The softer flooring available on the outdoor pack may also have increased oestrus duration; this interpretation is consistent with Britt et al. (1986) who found that oestrus duration was longer when cows were observed on dirt compared to on concrete.

Oestrus duration in our study was also influenced by DIM, a finding that contrasts that of Stevenson et al. (2014) who found no relationship between oestrus duration and DIM. In addition, oestrus duration increased with the number of cows in heat, a result that is in line with previous work (Hurnik et al., 1975; Van Vliet and Van Eerdenburg, 1996). Other studies also reported oestrus duration to be influenced by other factors, such as parity (Roelofs et al., 2005; Talukder et al., 2014) and milk production (e.g. Lopez et al., 2004; Talukder et al., 2014; Madureira et al., 2015). However, the definition of oestrus duration often varies between studies, which makes comparisons of this variable difficult between studies.

Seven of the 11 animals showing silent heats, showed these during their first oestrus event. Cows experience a negative energy balance in the weeks after calving (Beam and Butler, 1999) which may explain the reduced motivation to engage in energy-costly behaviours such as mounting. In addition, postpartum ovulations are often characterized by low oestradiol production, inhibiting oestrus expression (Allrich, 1994).

Our study differed from previous work in that we allowed cows to choose between indoor and outdoor environments over multiple oestrus events. Previous work did not provide cows a choice between different areas (e.g. Palmer et al., 2010; 2012), or only let cows choose for a limited time (Vailes and Britt, 1990). Our study was also the first to investigate the effect of providing cows access to an outdoor pack on oestrus expression when both OUTDOOR and INDOOR cows were kept in the same pen by means of an automated selection gate. One strength of this approach is that all cows experienced the same physical and social indoor environment, allowing treatment to be applied at the cow level, decreasing the number of cows required for the study. The chosen experimental setup also came with some limitations. The chances of a concurrent estrual cow being present was higher indoors than outdoors, given that there were always a minimum of 18 cows housed indoors. This may explain the lack of an overall difference in oestrus intensity between INDOOR and OUTDOOR treatments. In addition, we lacked power to analyse the observed oestrus behaviours separately. Other studies (e.g. Sveberg et al., 2011; Palmer et al., 2012) differentiated between various oestrus behaviours, such as standing to be mounted events, successful and unsuccessful mounts and disoriented mounts. However, it can be argued that these studies inappropriately treated individual cow, within a group, as the experimental unit.

5.5 Conclusions

Increasing indoor THI decreased oestrus intensity of INDOOR cows, but this effect was not present in OUTDOOR cows. OUTDOOR cows showed a lower oestrus intensity in the freestall barn with increasing indoor THI, but there was no effect of indoor THI on oestrus intensity expressed on the outdoor pack. The number of cows in heat also increased oestrus intensity. We conclude that access to an outdoor open pack facilitates the expression of oestrus behaviours in Holstein cows, especially under warmer environmental conditions.

Chapter 6: General discussion and conclusions

6.1 Findings and implications

In chapter 1, I set out to review the existing literature on how tie- and free-stall housing influence dairy cattle behaviour and how this compared to when cows are kept on, or given access to, pasture or another type of outdoor area. The existing literature indicated that pasture can facilitate the expression of several behaviours in dairy cows, such as feeding (i.e. grazing) (e.g. Redbo, 1990; Kilgour, 2012), lying (e.g. Krohn and Munksgaard, 1993; Ketelaar-De Lauwere et al., 1999; Legrand et al., 2009), standing (e.g. Hernandez-Mendo et al., 2007; Legrand et al., 2009) as well as social (Tresoldi et al., 2015) and oestrus behaviours (e.g. Palmer et al., 2010, 2012), compared to indoor housing. Some work on indoor open pack systems was available, indicating some benefits for lying and standing behaviour (Fregonesi et al., 2009b), but studies on the effects of open packs on social and oestrus behaviours were limited. In addition, no studies have investigated dairy cow preference for outdoor open packs. Identifying these knowledge gaps set the stage for my dissertation work, starting with the investigation of dairy cow preference for different types of outdoor access during the night, as described in Chapter 2.

When given free choice access during the night, dairy cows showed a strong preference for pasture over a sand pack, spending ~ 90% of the night on the pasture and only 1% of the night on the sand pack (see Chapter 2). Given that the primary aim of this experiment was to test the preference of dairy cows for outdoor areas that could potentially be implemented on commercial dairy farms, by design, the space allowance on pasture was much larger (i.e. 1750 m₂/cow) than on the outdoor sand pack (12 m₂/cow). However, this also represents an important limitation of the study, as the driving force for cow preference for pasture over a sand pack may simply have been due to there being more space on the pasture.

In Chapter 3, I investigated dairy cow preference for an outdoor wood-chip pack during the day and night. I initially ran this study during the summer and then replicated it during the winter. Cows especially spent time outside during summer nights but spent very little time outside during the winter. Longitudinal studies during multiple seasons are needed to better understand how dairy cow preference for outdoor access varies between seasons.

In Chapter 4, I tested whether outdoor space allowance influenced dairy cow behaviour and preference to access the outdoor-bedded pack. Dairy cow preference for an outdoor pack was positively influenced by increasing space allowance, but only during the night. The lack of an effect during the day was likely driven by cows spending very little time outside during the day. Although I had hypothesised more agonistic interactions on the bedded pack with decreasing space allowance, I did not find this. This finding may be explained by the cows preferring to spend more time indoors as a way to avoid negative interactions on the outdoor pack at the lower space allowances. Future studies should monitor the effects of social status of individual cows in these types of studies. Although dominance status in dairy cows is resource dependent (Val-Laillet et al., 2008), it is possible that lower ranking cows spent less time outdoors because they felt more vulnerable in the open area outside.

In Chapter 5, I showed that providing free-stall housed cows access to an outdoor pack can positively impact the expression of oestrus behaviour. The experimental design used here, i.e. that cows within a pen were randomly assigned to outdoor access or not, is a strength of the study, as this way all cows were exposed to the same physical and social environment indoors. This design enabled me to use 'cow' as the experimental unit, rather than 'group', which enabled the study to be completed in a timelier manner and with fewer cows than when treatment had to be applied on 'group' level. This approach has, to the best of my knowledge, not been previously used when investigating dairy cow preferences for different types of environments. Given the effectiveness of this experimental design, I would encourage future research to consider a similar approach.

A strength of all my studies is that cows were provided a *choice* to access an alternative outdoor area from the free-stall pen, i.e. outdoor packs were used to provide free-stall housed cows with additional space. To the best of my knowledge, this approach is novel, as previous work investigating dairy cow behaviour on in- or outdoor open pack systems (e.g. Fregonesi and Leaver, 2002; Boyle et al., 2008) or outdoor areas (e.g. Fisher et al., 2003; Schütz et al., 2015) did not provide cows the option to access an alternative area. Given that dairy cow behaviour may change depending on whether outdoor access is presented as a choice (e.g. Legrand et al., 2009), it is important to differentiate between choice and forced systems, including how each of these systems impacts animal welfare. Providing a choice between different environments allows the animal to exercise some control (agency) over their environment; now viewed to be an important component of animal welfare (Špinka and Wemelsfelder, 2011; Franks and Higgins, 2012; Špinka, 2019; Franks, 2019). Hence, the choice to go outdoors, rather than *keeping* cows outdoors, is of particular importance in terms of improving dairy cattle welfare. As such, the importance of providing cows a choice between different (indoor and outdoor) environments is likely to become increasingly important.

One question that could not be addressed in this thesis is how to integrate the bedding used for the outdoor pack into the life cycle of a dairy farm (Kristula et al., 2010). Several systems have been developed to separate sand used in free-stall housing from manure, which allows the sand to be recycled as bedding; a management practice that could also be implemented when using outdoor open packs. When a wood by-product is used as bedding, composting may be a more practical approach (Larney et al., 2008). Clearly, there will be regional differences in bedding availability

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which must be considered when identifying what types of bedding to use on outdoor bedded packs. Research addressing the environmental sustainability of different bedding types is a critical component that must be addressed when exploring outdoor open packs.

Another important question that remains to be investigated is the importance of outdoor access for dairy cows in of itself. Given that the indoor and outdoor environments available to the cows differ depending on the housing system provided (i.e. lying stalls versus of an open lying area, concrete flooring versus a deep-bedded sand pack, feed availability versus no feed provision outside, etc.) in my experiments, research is needed that investigates dairy cow preference for an indoor versus an outdoor open pack to understand the importance of outdoor access per se. For example, a study on the preference of dairy cows for a similar sized indoor versus outdoor open pack would be a valuable addition to the literature.

6.2 **Recommendations for future research and directions**

To allow a viable future for the dairy industry, it is crucial that dairy farming practices are acceptable to the public (Barkema et al., 2015). Given that the public is especially concerned about the natural living aspect of animal welfare (von Keyserlingk and Weary, 2017; Beaver et al., 2019), it is important for the dairy industry to effectively address this concern.

The dairy industry in North America has made attempts to address public concerns regarding dairy cattle welfare using animal welfare assurance schemes and corporate specifications. In North America, the two most common industry-led assurance programs are the Canadian Pro-Action program based on the Canadian Code of Practice for Dairy Cattle (NFACC, 2009) and the American Farmers Assuring Responsible Management (FARM) program (NFMP, 2016). Both programs are heavily focused on health and biological functioning parameters. In the

case of ProAction, there are guidelines related to pain mitigation and hunger (i.e. affective states) (NFACC, 2009) but to date the FARM program has remained silent on these issues. Unfortunately, neither program makes any attempt to address natural living concerns, such as access to pasture. In addition to industry-led assurance programs, there are some 'Product-differentiation programs' (i.e. labelling programs) for dairy products. Organic programs (e.g. USDA Organic, 2014; https://www.ams.usda.gov/sites/default/files/media/Dairy%20-%20Guidelines.pdf) as well as the British Columbia **SPCA** based certified (https://spca.bc.ca/programsprogram services/certifications-accreditation/spca-certified/; SPCA, 2018) are examples of such programs. In addition to measures related to the health and biological functioning and affective states of the animals, these programs include some requirements related to natural living. For example, organic production standards in both Canada and the US include requirements for pasture access; the SPCA certified program requires that cattle have access to pasture or an alternative outdoor area and be provided with brushes to groom themselves (Table 6.1).

Table 6.1 provides some examples of assurance programs and what type of animal welfare measures are emphasized in each of these. Neither proAction nor FARM emphasize the importance of outdoor access, despite much public concern around natural living evolving around pasture, or outdoor, access (e.g. Boogaard et al., 2008; Schuppli et al., 2014; Ventura et al., 2016; Cardoso et al., 2016; Hötzel et al., 2017). These latter studies also show that natural elements such as fresh air and the ability to roam, aspects that extend beyond pasture, are also often emphasized by members of the public (e.g. Schuppli et al., 2014; Ventura et al., 2016). Hence, given the current disconnect between North American dairy industry practices regarding outdoor access, I strongly recommend the inclusion of such measures in industry-led welfare assurance programs.

However, before these measures can be successfully implemented, farmers need to be encouraged to provide cows outdoor access. One option is to provide incentives to farmers willing to provide outdoor access. These price incentives could increase the number of cows that are provided outdoor access. For example, some European dairy companies have adopted these milk price regimes (Reijs et al., 2013) to encourage farmers to increase the use of pasture. In The Netherlands, the milk processor Friesland Campina pays members an additional 1.5 Eurocent per kilogram milk if they allowed cows pasture access for a total of 6 hours per day, a minimum of (https://www.frieslandcampina.com/nl/duurzaamheid/mvo-in-de-120 davs of the year praktijk/weidegang-koeien-in-de-wei/; FrieslandCampina, 2019). These initiatives have been argued by some to be instrumental in halting the trend towards reduced pasture access in The Netherlands (Duurzame Zuivelketen, 2018). I encourage that similar incentives be investigated in North America. These types of price incentives can be adapted to provide incentives for farmers to use bedded outdoor packs, particularly in environments where pasture is not feasible. Given the strong preference of dairy cows for pasture over an outdoor sand pack (Smid et al., 2018), and the feasibility of alternative outdoor areas compared to pasture, price incentives may need to vary with type of outdoor area provided. In addition, public acceptance of providing cows access to outdoor bedded packs in lieu of pasture will also determine the feasibility of these alternatives and I encourage research in this area. Given that dairy cow preference for outdoor access is influenced by weather (e.g. Legrand et al., 2009; Charlton et al., 2014; Smid et al., 2018, 2019) and by internal factors such as milk production and BCS (Charlton et al., 2011b), the provision of choice to access the outdoors seems to be important; I encourage welfare assurance programs to include the provision on choice in their recommendations.
Given that alternative outdoor areas such as the outdoor packs do not allow cows to graze, further work is needed on grazing motivation as this will provide context when evaluating the acceptability of alternative outdoor areas. As outlined in Chapter 1, grazing is potentially an important behaviour that cows are likely highly motivated to engage in. Chapter 2 also provides evidence indicating a strong preference of dairy cows for pasture over an outdoor sand pack, which may be related to a motivation of dairy cows to graze.

Given that the experimental designs used throughout this dissertation were designed to allow for inferences on behaviour, I was not able to investigate the effects of access to an outdoor open pack on dairy cow health. Lameness has been and remains one of the most important health problems for dairy cows (Webster, 1986; Wells et al., 1998; Ito et al., 2009). Lameness prevalence is often found to be lower in systems that allow cows access to pasture as compared to zero-grazing systems (Haskell et al., 2006; Olmos et al., 2009; de Vries et al., 2015; Adams et al., 2017) but not always (Bran et al., 2018). In the latter study, an important factor influencing lameness incidence was the walking speed with which cows were brought from pasture to the milking parlour, which was thought to be reflective poor animal handling skills. Ranjbar et al. (2016) found higher lameness prevalence in pasture systems with suboptimal handling of cows, associated with causing cows to push past each other on the track. Other factors such as quality of the track used between the pasture and parlour also influences lameness prevalence in pasture systems (Burow et al., 2014). Providing free-stall housed lame cows with a relatively short period on pasture (i.e. 4 weeks) allowed lameness to improve (Hernandez-Mendo et al., 2007). One reason for these beneficial effects may be that grass provides a softer standing surface than the concrete flooring inside the barn, allowing cows an opportunity to recover. Indoor open pack systems, that also provide softer standing surfaces, are also associated with lower rates of lameness than free-stall

systems (Haskell et al., 2006; Lobeck et al., 2011) and generally, pasture (Rutherford et al., 2008; Barrientos et al., 2013), or indoor open pack systems (Haskell et al., 2006; Lobeck et al., 2011) are associated with lower hock and knee lesion prevalence's compared to free-stall housing. Given that a deep bedded outdoor open pack provides cows with a soft surface to stand and lie on, and if well managed, has the potential to be a softer surface than pasture, providing cows access to such an area may be beneficial in reducing the risk of lameness.

Table 6.1 Dairy welfare assurance programs

The most commonly used dairy welfare assurance programs in North America and Europe, including their format (Fraser, 2006) and animal welfare concepts (Fraser et al., 1997) they address.

				Annai wenare concept		
Name protocol	Country of	Year	Format	Health and	Natural living	Affective
	origin			Functioning		states
Animal Welfare Approved	United States	2015	Product-Differentiation Program	+	+	+
Certified Humane	United States	2014	Product-Differentiation Program	+	+	+
Dairy Well	United States	2017	Corporate Specifications	+	-	+
FARM	United States	2016	Industry-led assurance program	+	-	+
Organic Production Systems	Canada	2015	Product-Differentiation Program	+	+	+
ProAction	Canada	2017	Industry-led assurance program	+	-	+
SPCA Certified	Canada	2018	Product-Differentiation Program	+	+	+
USDA Organic	United States	2018	Product-Differentiation Program	+	+	+
Welfare Quality ®	The Netherlands	2009	Research*	+	+	+
assessment protocol for						
cattle						

*The format 'research' was not specified by (Fraser and Koralesky, 2017) but is described by these authors as: 'a multi-institution research and development project sponsored by the European Commission'.

6.3 Conclusions

In this thesis, I have provided a body of evidence that indicates that dairy cattle have a partial preference to access an outdoor deep-bedded open pack, especially during summer nights. Cows also spent more time outside with increasing outdoor space allowance during the night. In addition, an outdoor open pack facilitates the expression of oestrus behaviour. When given a choice, cows preferred a pasture over an outdoor sand pack. As cow preference for the outdoors depends on many internal and external factors, providing cows a choice to go outdoors seems of particular importance.

Based on the results of this thesis, providing dairy cows access to the outdoors appears to be important. Pasture should be provided where feasible; on farms where pasture is not an option, an outdoor open pack can be implemented to increase dairy cattle welfare.

Future research should investigate the importance of grazing for cows. Given that cows have no ability to graze in outdoor open packs, insight into the importance of this behaviour for dairy cattle welfare is of utmost importance.

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