USING BEHAVIOUR TO IDENTIFY SICKNESS AND TO EVALUATE TREATMENT
IN ILL TRANSITION DAIRY COWS

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**USING BEHAVIOUR TO IDENTIFY SICKNESS AND TO EVALUATE TREATMENT IN ILL TRANSITION DAIRY COWS**

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Abstract

Dairy cows are at high risk of becoming ill with metabolic and inflammatory diseases during the transition period, considered the 3 weeks before and 3 weeks after calving. In many species, behavioural changes induced through the inflammatory process and modulated through neuro-endocrine pathways have been described, including for example, anorexia, decreased activity and decreased social interactions. Such changes in behavior have typically been referred to as ‘sickness behaviours’; an area of study that has seen increased interest in dairy cattle as a way of identifying sick cows and evaluating the effect of treatment. The goal of my thesis was to explore sickness-associated behavioural changes in transition dairy cows, and how these changes are affected by treatment. Chapter 1 summarizes current literature on the factors that contribute to the high disease risk for transition cows, followed by a summary on sickness behaviours in general, and in dairy cows specifically. Data for all research chapters were derived from one study. Chapter 2 examined the effects that treatment with a non-steroidal anti-inflammatory drug (meloxicam) had on the behaviour of dairy cows with metritis; the results did not reveal a clear benefit of meloxicam treatment. Chapter 3 described differences in behaviours at the lying stall between primiparous cows with metritis and healthy cows. The results showed that, in the 3 days before metritis diagnosis, cows with metritis spent more time standing fully in the stall and had more aborted lying events. Chapter 4 identified behavioural differences between cows with fever (but without clinical disease) and healthy cows with normal body temperature. The observed differences in this third study were in line with sickness behaviours previously described in other species; for example, cows with fever consumed less feed, spent less time feeding and engaged in fewer social interactions at the feed bunk. Collectively, the results of my thesis contribute to
our knowledge of sickness behaviours in dairy cows and support the idea that these behavioural changes can help to identify sick cows early and to evaluate treatment efficacy. The results may also provide a basis for targeted research on the environmental needs of ill cows.
Lay Summary

Many dairy cows become ill in the weeks around calving and experience compromised welfare. Illness may result in behavioural changes referred to as ‘sickness behaviours’. My thesis examines how dairy cows change behaviours at the feed bunk and in the lying area when ill and after being treated. The first study tests the effect of treatment with an anti-inflammatory drug on the behaviour of cows with uterine infection. The second study describes changes in behaviour at the lying stalls and associated with lying down in the days before cows were diagnosed with uterine infection. In the third study, changes in feeding and lying behaviours in cows with fever were examined. Overall, the findings of my thesis suggest that sickness behaviours can help to identify sick cows early, and to inform management practices that help ill cows recover.
Preface

All data for my thesis were derived from a single multiyear study that was conducted at the UBC Dairy Education and Research Centre in Agassiz, British Columbia, Canada. All animals were cared for following the guidelines of the Canadian Council on Animal Care (2009) and all procedures were approved by the UBC Animal Ethics Committee (Protocols A10-0163 and A14-0040). The study took place from July 2013 to October 2014. Throughout the thesis, I refer to the first person plural when referring to research developed and conducted in collaboration with co-authors. When expressing my own personal opinions, I use the first person singular.

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Huzzey collected the data. J. Lomb analyzed the data and wrote the manuscript. Co-authors aided with interpretation of the material and editing of the manuscript.

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List of Abbreviations

BCS = Body Condition Score  
BHB = β-Hydroxybutyrate  
BW = Body Weight  
CI = Confidence Interval  
COX = Cyclooxygenase  
DIM = Days in Milk  
DM = Dry Matter  
DMI = Dry Matter Intake  
LPS = Lipopolysaccharide  
NEB = Negative Energy Balance  
NEFA = Nonesterified Fatty Acids  
NSAID = Non-Steroidal Anti-Inflammatory Drug  
PGE$_2$ = Progesterone E$_2$  
SARA = Subacute Ruminal Acidosis  
s. c. = subcutaneous  
TMR = Total Mixed Ration  
TNF-α = Tumor Necrosis Factor-α
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Chapter 1: Introduction

1.1 General Introduction

Typically once a year, a dairy cow gives birth to a calf. This coincides with a period of tremendous physiological change as she transitions from a non-lactating, pregnant state to lactation. The time around parturition is typically referred to as transition period, beginning three weeks before and ending three weeks after parturition (Grummer, 1995; Drackley, 1999). The physical and physiological demands placed on the cow during transition are frequently paired with changes in management such as moving to a new group and a change in diet. For the primiparous cow this also includes introduction to the milking parlor, as well as comingling with older cows for the first time. Perhaps because of this multitude of challenges the transition period is a time when dairy cows are at increased risk of becoming ill (LeBlanc 2006), also resulting in high rates of mortality and involuntary culling during the first 60 days of lactation (Dechow and Goodling, 2008). Not surprisingly, some have argued that transition cow disease is one of the greatest welfare challenges facing the dairy industry (von Keyserlingk et al., 2009).

Likely due to high disease incidence around parturition, to date research emphasis on transition cow welfare has focused almost exclusively on the concept of biological functioning and health (see von Keyserlingk et al., 2009); this also corresponds well with the views of farmers who often cite health and production as the primary factors associated with care of dairy cattle (Te Velde et al., 2002; Lassen et al., 2006; Verbeke, 2009). However, following Fraser et al. (1997), biological functioning is only part of what constitutes animal welfare, the affective states of an animal and their ability to perform natural behaviours are also important. These two latter areas may be compromised directly during transition; for example, parturition is painful
(Mainau and Manteca, 2011), and most housing systems prevent cows from natural self-isolation behaviours (Lidfors et al., 1994; Robichaud et al., 2016). In addition, the diseases common during transition (e.g. mastitis, metritis) are painful themselves (Stojkov et al., 2015; Boyers des Roches et al., 2017), or predispose the animal for ailments that are painful or require intensive interventions (e.g. metabolic disease increases the risk for metritis and displacement of the abomasum; Suthar et al., 2013). Disease may also hinder the cow’s ability to express natural behaviours; for example, hoof disorders resulting in lameness limit free locomotion and heat expression (Weigele et al., 2018).

Improving transition cow health may come about by prevention of disease and by early identification of ill cows allowing for timely intervention. Traditionally, research and industry have sought to improve both strategies through optimizing physiological adaptation of the cow to transition, e.g. by using nutritional strategies that improve metabolic health and immune function (e.g. Grummer, 2008; and Zebeli, 2015), and by using biological markers to identify cows with and at risk for metabolic and inflammatory disease (e.g. Ospina et al., 2010; Shin et al., 2018). Despite these efforts, disease rates in transition cows remain high. A newer approach to identify preventative management strategies and to detect ill cows early is the use of behaviour and behaviour change (Weary et al., 2009, Sepulveda-Varas et al., 2013). This approach is becoming increasingly relevant, as technologies that allow for better monitoring of these behaviours are becoming widely available on farms.

In this introductory chapter, I present a brief literature review on transition cow health, including relevant challenges to the transition cow that contribute to the high incidence of disease. I also review the literature on sickness behaviours in cows and their use to identify sick
cows. I conclude this chapter with the specific research questions that guided the research of my thesis.

1.2 Transition Cow Health

1.2.1 Risk factors for transition cow illness

1.2.1.1 Transition physiology and nutrition

Energy demands on the cow are high during the transition period, with the main sources for energy expense being for fetal growth during late gestation, followed by colostrogenesis and lactogenesis after parturition (Bell, 1995). Every mammal goes through a period of negative energy balance (NEB) after giving birth where metabolic demands associated with lactation exceed dietary energy intake. The challenge is when this period is extended (Grummer et al., 2004) and the mobilization of body fat, to support gluconeogenesis in the liver (Herdt, 2000), results in metabolic dysfunction. This metabolic challenge is especially problematic for high yielding dairy cows (Ingvartsen et al., 2003; Oltenacu and Broom, 2010).

The metabolic demand in itself is a risk factor for disease, as the resulting metabolic stress impairs the functioning of other physiological processes, such as the immune (Zerbe et al., 2000; Hammon et al., 2006) and reproductive system (Chagas et al., 2007; Aleri et al., 2016). Consequently, incidences of metabolic and inflammatory disease have been reported higher in cows that were metabolically challenged, as evidenced by elevated metabolic blood parameters (Ruprechter et al., 2018) and by loss of body condition during the dry period (Chebel et al., 2018).

The nutritional management of transition cows may further aggravate the risk for cows to become ill. First, the fed diet may not provide a transition cow with sufficient energy, thus
increasing the risk for a prolonged NEB (Grummer, 2008) and risk of metabolic disease (Overton and Waldron, 2004). Further, diet composition may provide the cow with sub-optimal levels of nutrients important for immune functioning (Sordillo et al., 2016). Lastly, energy dense diets carry the risk for lowering ruminal pH and result in sub-acute ruminal acidosis (SARA; Humer et al., 2018).

Management related to the diet may contribute to the health status of the cow as she proceeds through the transition period. For example, the time relative to calving when necessary dietary changes are made may further impact how well cows are able to cope. Recent work has shown advantages to maintaining cows on a low energy diet for the entire dry period, instead of switching to a high energy diet in the weeks prior to calving (Vickers et al., 2013; Zhang et al., 2015). Other management strategies have been associated with changes in feeding behaviours, including reductions in DMI. For example, regrouping has been associated with reduced feeding time and DMI (Schirmann et al., 2011) which may increase the risk for insufficient energy intake. Providing reduced feeding space per animal (often referred to as overstocking) at the feed bunk has been shown to increase competitive behaviours (Proudfoot et al., 2009b; Miltenburg et al., 2018) leading to increasing within-group variability of feeding patterns (Crossley et al., 2017) which may in turn lead to individual cows consuming different proportions of forage and concentrate as result of feed sorting. The effects of management strategies associated with behavioural changes in transition cows discussed in more detail in section 1.2.1.2.

Some argue that, other than the discussed metabolic challenges, a prolonged inflammatory response may also result in an increased risk for peri-parturient disease for transition cows. Increased concentration of inflammatory markers (i.e. acute phase proteins) in the days after parturition have repeatedly been observed in clinically healthy cows (Huzzey et
al., 2009; Bossaert et al., 2012; Burfeind et al., 2014). This inflammatory response may be provoked by endotoxins that are released by bacteria of the gastro-intestinal system (e.g. cows with SARA), the uterus and the mammary gland, and in some cases may predispose cows to clinical disease (Eckel and Ametaj, 2016). Given that mounting an immune response is energetically costly and requires reallocation of resources to provide energy to immune cells (Bradford et al., 2009, 2015; Kvidera et al., 2016), the initiated immune response may in turn contribute to the metabolic challenge faced by the transition cow.

1.2.1.2 Management

Throughout lactation, cows are typically grouped depending on their lactational stage and nutritional needs. In the peripartum period, when cows transition from gestation to lactation, each change is accompanied by a change in diet. Consequently, pen moves are frequent and cows are forced to regularly comingle with unfamiliar conspecifics (see Cook and Nordlund, 2004). In a recent survey of dairy farmers in the United Kingdom, 84 % reported that they housed dry cows in dynamic social groups with regular introductions of new cows to the group (Fujiwara et al., 2018). Moreover, overstocking, i.e. a higher number of cows than spaces, is not uncommon in confinement dairy herds (Endres and Espejo, 2010; Sova et al., 2013), and 14 % of UK farmers reported that not all pre-partum cows in their herd could access feed at the same time (Fujiwara et al., 2018).

Both overstocking and regrouping have been shown to alter behaviour in transition dairy cows. Transition cows at higher stocking density have more agonistic interactions at the feeder (Proudfoot et al., 2009b; Lobeck-Luchterhand et al., 2015; Miltenburg et al., 2018), and have shorter lying times (Lobeck-Luchterhand et al., 2015; Miltenburg et al., 2018). Proudfoot et al.,
(2009b) also reported that cows that were displaced most often ate at a faster rate. Such
behavioural changes may impact health directly and further on in lactation. For example, a faster
feeding rate may increase the risk for ruminal acidosis given that faster feeding leads to lower
salivation rates (Beauchemin et al., 2008), and prolonged standing before calving has been
associated with an increased risk for sole lesions postpartum (Proundfoot et al., 2010).

Regrouping may also result in behavioural changes. When investigating the effects of
regrouping multiparous cows in the dry period, Schirmann et al. (2011) found that moved cows
showed greater behavioural changes than cows that remained in the pen; cows that were
introduced decreased dry matter intake (DMI) and were replaced more often by other cows at the
feeder. Both, cows that were moved and those that remained decreased feeding time and feeding
rate in the days after regrouping (Schirmann et al., 2011). Some effects of regrouping may be
different depending on the group size the moved cow is introduced to (Jensen and Proudfoot,
2017); cows that were moved to a group with fewer cows showed less agonistic interactions than
cows introduced to a larger group, while cows of both groups increased feeding and lying
behaviours over time. Jersey cows that were housed in either stable groups (control) or groups
subjected to weekly regrouping showed also more displacements at the feed bunk in the
regrouped pens (Lobeck-Luchterhand et al., 2014). In the same study, the control cows had
shorter feeding times in the first week of the study, but appeared to rebound given that they had
longer feeding times in week two and three of the study. Clear behavioural changes in feeding
patterns were apparent between pre-partum cows housed in a predictable and highly
unpredictable environment, where they were overstocked and regrouped, fed at different times,
and assigned a new feeding bin every second day (Proudfoot et al., 2018); cows in the
unpredictable environment had longer but fewer visits to the feeder, spent less time feeding and were involved in more agonistic social interactions.

Collectively these lines of research provide evidence that common on-farm management practices have negative impacts on transition cow behaviour. Some studies also described effects of such management strategies on metabolic and immune system measures. Proudfoot et al. (2018) reported effects of the unpredictable environment on health outcomes, as cows housed in this challenging environment pre-partum had higher non-esterified fatty acids (NEFA), higher tumor necrosis factor (TNF)-α and more cows were diagnosed with cytological endometritis. Results of another study suggested that cows, when overstocked at the feeding and lying area for 14 consecutive days during the dry period, were likely metabolically compromised as they had greater NEFA concentrations and showed a tendency for greater fecal cortisol concentrations (Huzzey et al., 2012b). The latter has been associated with increased risk for disease and death post-partum (Huzzey et al., 2011). Similarly, Fustini et al. (2017) reported increased cortisol and dehydroepiandrosterone (i.e. a steroid suggested to be indicative of stress, Sporer et al., 2008) in pre-partum cows housed in an open pack with restricted lying and feed bunk space. However, these metabolic markers were only elevated for a single day close to start of the study, questioning the biological relevance. In contrast, another study (Miltenburg et al., 2018) found that cows in understocked (80 % stocking density) and overstocked (120 % stocking density) groups throughout transition had only moderate differences in some metabolic and inflammatory markers. Moreover, there were no differences in metabolic or inflammatory markers or health outcomes between pre-partum Jersey cows housed at different stocking densities (80 % vs. 100 %; Silva et al., 2016) or in stable versus dynamic groups (Silva et al., 2013 a, b). In contrast, Kaufman et al. (2016) reported greater odds for cows to be diagnosed with subclinical ketosis
with increasing stocking density in the lying area. These authors, however, did not identify lying behaviour as a risk factor for subclinical ketosis (as was reported by Itle at al., 2015), and failed to report other management factors that may have influenced the outcomes of their study, such as stocking density at the feed bunk.

1.2.1.3 Social Stress

A number of behavioural changes in response to challenging management practices like overstocking and regrouping, e.g. an increase in agonistic behaviours representing increased competition for a given resource, can also be interpreted as sign for social stress (Proudfoot and Habing, 2015). These management practices may not affect all animals equally but rather be more challenging for some individuals within a group, possibly putting them at higher risk for becoming ill. One determinant of the extent to which the environment negatively impacts an individual cow is her social status within a group. A cow’s social status is often defined by the ratio of successful and unsuccessful competitive interactions, described as displacements (i.e. number of cows she displaces/ (number of cows she displaces + number of cows that displace her) *100; based on index of success: Mendl et al., 1992). Based on this index of success, a social hierarchy can be established and cows classified as high-, middle-, and low-ranking in regard to this resource (Galindo and Broom, 2000). Given that most competitive interactions in freestall housed dairy cows take place at the feeder (Val-Laillet et al., 2008), most studies have used this resource to measure displacements and calculate social hierarchy.

There is some evidence that cows of low social rank may be more affected by a challenging environment. Lactating cows with a lower rank at the feed bunk appear to benefit most from less competitive environments at the feeder (DeVries et al., 2004, Huzzey et al.,
When assessed in the dry period, low ranking cows appeared to have a higher stress response, measured as fecal cortisol, than higher-ranking cows (Huzzey et al., 2012a). In addition, primiparous but not multiparous cows housed in mixed-parity groups showed increased cortisol levels in conditions with limited access to the feed bunk (Huzzey et al. 2012b). First parity cows are likely to be of lower social rank when housed together with multiparous cows (Gonzalez et al., 2003).

Given our knowledge of the effects of psycho-social stress in humans (e.g. Glaser et al., 1992; Segerstrom and Miller, 2004) and laboratory rodents (e.g. Hennessy et al., 2010), we can expect social stressors to negatively impact immune function and health in transition dairy cows, especially those of low rank, but to date few studies have addressed this issue (Proudfoot and Habing, 2015; Chebel et al., 2016). When housed at 80% stocking density, pre-partum cows with high and middle competitive success had greater immune function (measured as oxidative burst) than cows with little success (Miltenburg et al., 2018). Moreover, transition cows that engaged in fewer competitive interactions at the feeder in the pre-partum period, possibly of low social rank, were at greater risk for metritis (Huzzey et al., 2007) and subclinical ketosis (Goldhawk et al., 2009) after parturition. Social rank during transition also affected hoof health later in lactation; low- and high-ranking cows were more likely to develop hoof lesions than middle ranking cows (Proudfoot et al., 2010).

Together, these findings may explain why transition cows that engaged in fewer competitive interactions at the feeder in the prepartum period were diagnosed with metritis (Huzzey et al., 2007) and subclinical ketosis (Goldhawk et al., 2009) after parturition; in both studies, cows were overstocked at the feed bunk (20 cows with access to 12 feeders). Compared with healthy cows, those diagnosed with metritis (Patbandha et al., 2012) and clinical ketosis
(Ile et al., 2015) were shown to have longer standing times before calving, suggesting that social rank may have played a role in low ranking animals having less access to the feed bunk, which resulted in them having to stand and wait to gain access.

1.2.2 Transition cow disease

1.2.2.1 Common transition cow diseases

The numerous stressors that negatively affect behaviour, metabolic functioning and immune functioning ultimately contribute to cows being at increased risk of becoming ill during the transition period. Some argue that about 30 – 50 % of all cows become ill during this time (LeBlanc, 2010). Among the most frequently reported metabolic diseases are subclinical and clinical hypocalcemia (Goff, 2008; Mulligan and Doherty, 2008; Reinhardt et al., 2011) and subclinical and clinical ketosis (Suthar et al., 2013; Tatone et al., 2017). Common infectious diseases observed in the weeks following calving include metritis (Giuliodori et al., 2013; Vergara et al., 2014; Pohl et al., 2016) and mastitis (De Vliegher et al., 2012). In addition to clinical disease, a high proportion of transition dairy cows show at least one day of fever during the first 10 days in milk (DIM; Wenz et al., 2011: 14% in primiparous cows; 15 % in multiparous cows; Suthar et al., 2012: up to 46.8 % during high ambient temperatures), possibly in response to an undiagnosed infection or tissue damage.

Given that much of the research of my thesis focused on dairy cows with metritis, I conclude this first part of the introduction with a short summary on this malady.

1.2.2.2 Metritis

Metritis, the inflammation of all layers of the uterus (Lewis 1997) is one of the most common ailments in transition dairy cows, with incidences of 30% or higher (Giuliodori et al.,
2013; Vergara et al., 2014; Pohl et al., 2016). The inflammation is caused by a range of non-specific, environmental gram-positive and gram-negative bacteria including *Fusobacterium necrophorum* and *E. coli* (e.g. Kasse et al., 2016; Cunha et al., 2018). These and other bacteria are found in the lumen of the uterus of almost every cow after calving (Sheldon et al., 2002; Földi et al., 2006). Development of clinical metritis depends on the presence of risk factors such as virulence factors of bacteria (Bilcalho et al., 2012) and several cow-level risk factors (Sheldon et al., 2008) including dystocia and retained placenta (Bruun et al., 2002; Dubuc et al., 2010b), vulvovaginal injury resulting from calving (Viera-Neto et al., 2016), parity (cows in first or over 3rd lactation at higher risk: Bruun et al., 2002; first parity cows at higher risk: Mahnani et al., 2015), decreased metabolic functioning (Hammon et al., 2006, Dubuc et al., 2010b, Suthar et al., 2013) and decreased DMI and feeding time in the week before parturition (Huzzey et al., 2007).

Metritis is typically diagnosed within 21 DIM (Sheldon et al, 2006) and the diagnosis based on uterine discharge, collected vaginally, and interpreted alone or in combination with complementary clinical symptoms. Sheldon et al. (2006) differentiates between two forms of metritis, where (1) cows show signs of systemic illness (e.g. depression, anorexia, fever) in addition to an enlarged uterus and watery, fetid discharge (i.e. ‘puerperal’ metritis), or (2) cows show no other signs than purulent vaginal discharge (i.e. ‘clinical’ metritis). Others (Urton et al., 2005; Huzzey et al., 2007) base metritis diagnosis solely on the quality of vaginal discharge, categorizing cows into one of five possible scores, with scores 0 and 1 indicative of a healthy uterus, and scores 2-4 indicative of uterine inflammation (0 = clear mucus, no smell; 1 = mucus with flecks of pus, cloudy or bloody mucus, no smell; 2 = mucus with <50% pus and foul smell; 3 = mucus with >50% pus and foul smell; 4 = watery, brownish discharge with foul smell).
Farmers perceive metritis to be painful for cows (Thomsen et al., 2012), and recent studies support this perception. Cows with metritis reacted with a more pronounced back arch and increased heart rate variability to passive rectal palpation and to uterine palpation, suggesting that they were feeling visceral pain (Stojkov et al., 2015). Cows with metritis also had elevated concentrations of Substance P (Barragan et al., 2018), a physiological marker associated with pain. In addition, metritis has been associated with behavioural changes in line with sickness behaviours (Huzzey et al., 2007; Schirmann et al., 2016; discussed in detail in section 1.3.2.) suggesting a feeling of malaise (Weary et al., 2009) in affected cows. Uterine inflammation also has negative implications from an economic viewpoint, as it is often followed by a prolonged phase of uterine involution (Heppelmann et al., 2013), increased risk of endometritis (Cheong et al., 2011), ultimately leading to reduced reproductive performance (Fourichon 2000; Elkjaer et al., 2013). Metritis has also been associated with reduced productive performance (Mahnani et al., 2015; Piccardi et al., 2016).

Antimicrobials, in particular ceftiofur, are deemed to be effective metritis treatment (Haimerl et al., 2017) and are commonly used on farms (Zwald et al., 2004, Espadamala et al., 2018). Treatment with non-steroidal anti-inflammatory drugs (NSAID), alone or in combination with antimicrobials, had varied effects on productive and reproductive performance of metritic cows (Amiridis et al., 2001; Drillich et al., 2007; Pohl et al., 2016). Preventative strategies have also been investigated, including vaccination against pathogens commonly associated with metritis, but results varied (Machado et al., 2014; Freick et al., 2017). Other less investigated preventative treatment options include the use of intra-vaginally administered probiotics (Otero et al., 2006; Genis et al., 2017) and intra-vaginal treatment with bacteriophages (Machado et al., 2012; Meira et al., 2013).
Interestingly, much of the research concerning medical therapy of metritis has focused on the effect of the tested drug on production and reproductive measures. Even when testing the effect of an NSAID, a drug with known analgesic effects, the focus was productive and reproductive measures and immediate clinical outcome measures were limited to antipyretic effects (Pohl et al., 2016) as opposed to consideration of measures that indicate improved well-being of the cow. Assessment of behavioural measures, as discussed in the following section, may help fill this gap by evaluating treatment efficiency as experienced by the cow.

1.3 Using behaviour to assess transition cow health

1.3.1 Sickness behaviours

In 1988, Hart described the suite of behaviours that are typically observed in ill humans and animals, and argued that these behaviours were not simply an outcome of the individual’s debilitation, but rather a strategy that supports the mounting of a fever and overall immunological defense system of the organism at a ‘life-or-death-juncture’. Behavioural responses now described in many vertebrate species include anorexia, adipsia, depression and reduced activity, increased resting and avoidance of social interactions (Hart, 1988; Dantzer and Kelley, 2007), and are thought to be associated with a feeling of malaise (Weary et al., 2009).

The magnitude to which sickness behaviours are expressed has been shown to vary with environmental and individual specific factors, for example social context (Hennessy et al., 2014) and distress (Avitsur et al., 2013).

Fever and sickness behaviours are modulated through neuro-endocrine pathways induced by infectious or non-infectious (e.g. tissue damage) inflammation. Pro-inflammatory cytokines (e.g. interleukin-1 and -6 and TNF α) are produced by the innate immune system at the
inflammation site and, ultimately, lead to increase of prostaglandin E2 (PGE$_2$) in the central nervous system (see Pecchi et al., 2009 for full review on pathophysiological pathways). Central PGE$_2$ is thought to be the primary hormone that induces fever and sickness behaviours. The expression of fever and sickness behaviour appears to be dose-dependent; in rats infected with brewer’s yeast fever as well as sickness behaviour were expressed to a greater extent with increased dose and lasted for a longer time (Dangarembizi et al., 2017). However, fever and sickness behaviour are not always expressed simultaneously. Mice of different species varied in their response to LPS, in that some mounted a fever response (those that were capable of killing bacteria in vitro), and others expressed more sickness behaviours but did not mount a fever (Martin et al., 2008).

While it is widely agreed upon that fever and sickness behaviours are beneficial to the host organism, especially how sickness behaviours support the immune system is not yet well understood. Fever has been shown to enhance functioning of the innate immune system rather than inhibiting pathogen growth directly (Blatteis, 2003). Sickness behaviours were first thought to primarily support the energetically costly fever response by conserving energy (i.e. by increasing resting time) and to reduce support for pathogen proliferation (i.e. by inducing anorexia and subsequent lowering of blood iron concentrations; Hart 1988). Others now argue that a primary function of sickness behaviours may be to limit pathogen distribution within a population (Shakhar and Shakhar, 2015). On the other hand, research in some species (e.g. birds, guinea pigs) has demonstrated that infected individuals expressed sickness behaviours when kept in isolation while suppressing them when in proximity to social partners (Hennessy et al., 2014). Together, these findings indicate that especially the social aspect and social influence on the expression of sickness behaviours may be complex and it is little understood in dairy cows.
1.3.2 Sickness behaviours in dairy cows

Few studies have investigated behavioural changes and fever in dairy cows after experimentally induced infection not associated with a specific disease. For example, dairy cows had increased concentrations of pro-inflammatory cytokines and decreased DMI after intra-jugular treatment with lipopolysaccharide (LPS; Zhao et al., 2018). Moreover, while reduced feed intake in the 24 h after LPS infusion was shown to be dose-dependent (i.e. greater decrease in feed intake with increasing LPS concentration administered), an increase in body temperature was less dependent on dose and rarely resulted in fevers about the commonly accepted threshold of 39.5°C (Waldron et al., 2003).

Other work investigated behavioural change in cows with experimentally induced mastitis through intra-mammary infusion of *E. coli* LPS. For example, early lactation primiparous cows infused with *E. coli* in one quarter decreased their feeding time for 24 h after infusion, but increased standing for 72 h when compared to their own baseline behaviours (Fogsgaard et al., 2012). In another study, cows which received intra-mammary *E. coli* LPS had decreased DMI (1 kg/d) on the day of infusion, but no overall decrease in daily rumination (Fitzpatrick et al., 2013). Fitzpatrick et al. (2013) also continuously assessed body temperature by use of electronic data loggers and reported that a fever response peaked at approximately 41°C after infusion, but that the response lasted less than 8 hours. Combined with the findings on cows challenged with jugular LPS, these results suggest that under experimental conditions, a single administration of infectious agent induces a rapid but short-lasting rise in body temperature and decline in feeding behaviour.

Dairy cows with naturally occurring (i.e. not induced) infectious diseases have also been reported to express sickness behaviours. A number of studies reported that cows with metritis
develop sickness behaviours, but results were presented as weekly averages, thus lacking in
detail on when sickness behaviours first occur in relation to clinical symptoms (i.e. altered
vaginal discharge). Cows with naturally occurring metritis had lower DMI, averaged by week,
than healthy cows in the first 2 (Schirmann et al., 2016) and 3 weeks postpartum (Huzzey et al.,
2007) and also spent less time at the feeder during the first 3 weeks after calving (Urton et al.,
2005; Huzzey et al., 2007). It is noteworthy that some of these behavioural differences between
healthy and metritic cows were already evident during the prepartum period (Huzzey et al.,
2007), suggesting that some of these behaviours should be considered risk factors rather than
cytokine mediated sickness behaviours.

Others, investigating sickness behaviours in cows with naturally occurring mastitis,
reported behavioural change on a daily basis and in temporal relation to the occurrence of
clinical symptoms. Cows with moderate mastitis (i.e. one quarter showing clinical symptoms,
presence of fever not mandatory) started to decrease in a number of feeding behaviours, i.e.
DMI, feeder visits, actor replacements and DMI during peak feeding times, 3 to 4 days before
visible changes in milk composition occurred (Sepulveda-Varas et al., 2016). In contrast, cows
with mastitis (diagnosed based on milk sample only and without consideration of body
temperature), had lower feed intake, lower feeding rates and also shorter lying times when
compared with healthy cows even 10 days before mastitis was diagnosed and treated (Fogsgaard
et al., 2015). Cows with naturally occurring mastitis, especially when caused by E. coli or when
diagnosed in addition to other disease, were also found to decrease rumination time and physical
activity for 1 and 5 days, respectively, before clinical diagnosis by farm staff (Stangaferro et al.,
2016a). In this study, rumination returned to baseline within 4 days after diagnosis, whereas
activity remained below the values of healthy controls.
While multiple studies have reported a decrease in activity in cows with infectious disease (i.e. mastitis and metritis; Stangaferro et al., 2016 a, b; Liboreiro et al., 2015), findings on lying behaviour have not been consistent. Compared with control cows, animals with metritis had increased lying times in the days after diagnosis (Barrigan et al., 2018). Similarly, primiparous cows diagnosed with a combination of multiple health disorders (i.e. metritis, retained placenta or mastitis) had longer lying times in the days after parturition, and tended to have longer lying bouts (Sepúlveda-Varas et al., 2014). However, cows with mastitis were consistently reported to increase standing times (e.g. Cyples et al., 2012; Fogsgaard et al., 2015). Together, these results suggest that reduced activity (i.e. movement, commonly measured as number of steps taken within a time period) may not always be associated with increased lying times in dairy cows, and that lying behaviours may be influenced by multiple motivations.

To date, changes in social behaviours as sign of sickness have garnered little attention in dairy cows, even though an integral part of the sickness response (Shakhar and Shakhar, 2015). Some of the behavioural changes observed at the feed bunk, e.g. decreased competition and reduced feed intake during peak feeding times, may well be a sigh for social disengagement as opposed to a decreased motivation to feed. To my knowledge, only one study has investigated social isolation more specifically and this study found that cows are motivated to isolate themselves when ill (Proudfoot et al., 2014); however, this study only followed 9 sick cows and more work is needed to substantiate this finding.

1.3.3 Pain behaviours in transition dairy cows

It has been well established that pain can lead to behavioural change (Weary et al., 2006), and some periparturient diseases that induce sickness behaviours, e.g. mastitis and metritis, have
also been associated with pain (mastitis: Fitzpatrick et al., 2013; Boyers des Roches et al., 2017, 2018; metritis: Stojkov et al., 2015). It follows that future work on using behavioural change as an indicator of disease must consider whether behavioural changes are a consequence of pain or sickness. Therefore, pain behaviours as described in transition cows are briefly discussed in the following paragraphs, even though not the focus of this thesis.

Cows experiencing eutocia (i.e. calving without assistance) show behavioural changes in the days and hours before calving, many of which have been associated with pain. Among the most frequently observed behaviours are an increase in number of transitions between standing and lying before calving (Miedema et al., 2011a; b; Jensen, 2012), and shorter lying times on the days and hours before parturition (Huzzey et al., 2005; Jensen, 2012) combined with increased activity (i.e. average acceleration per second, measured by an electronic data logger attached to the leg; Jensen et al., 2012). However, calving cows also express behaviours that resemble sickness behaviours, as they decrease feed and water intake (Jensen et al., 2012) as well as rumination time (Schirmann et al., 2013) in the hours before calving, and socially isolate when provided the opportunity (Proudfoot et al., 2014). Dystocia (i.e. parturition with difficulties requiring assistance), which in humans is reported to be associated with higher levels of pain compared with eutocia births (Eisenach et al., 2008), resulted in cows transitioning from standing to lying more often on the day before calving (Proudfoot et al., 2009a). These authors also reported that cows experiencing dystocia showed more sickness behaviours compared to eutocia cows; DMI was lower 48 h before parturition, accompanied by reduced water intake in the 24 h before calving assistance was given. A recent study also reported that cows with longer calving times and more abdominal contractions, likely resulting in a higher degree of pain, preferred to
calve in the space with the highest isolation level, while cows with eutocia did not show a clear preference (Rørvang et al., 2017).

In the case of some transition cow diseases, e.g. mastitis and metritis, it might not always be clear if behavioural changes are a consequence of pain or sickness. In both disease states sickness behaviours are expected, and have been reported, for example reduced DMI and feeding times (metritis: Huzzey et al., 2007; mastitis: Fogsgaard et al., 2015; Sepúlveda-Varas et al., 2016). However, both ailments may also be painful, as indicated by cows with mastitis showing avoidance of pressure placed on the mammary gland (Fitzpatrick et al., 2013) and cows with metritis showing a back arch in response to trans-rectal palpation of the uterus (Stojkov et al., 2015). In addition to these pain specific responses, animals may decrease certain behaviours they usually perform (see review by Weary et al., 2006), such as exploratory behaviour (Martin et al., 2004), and these may be similar to sickness behaviours. Further, some behavioural changes in response to pain may contradict known sickness behaviours; for example, cows with mastitis were repeatedly shown to increase standing time (Siivonen et al., 2011; Medrano-Galarza et al., 2012), despite sickness generally thought to increase resting (Hart 1988). These findings must be considered when evaluating sickness behaviours used for identification of disease. In addition, understanding the motivation for the behavioural change (i.e. sickness or pain) may be useful for developing treatment strategies.

One way of disentangling the effects of pain and sickness is comparing behaviours with and without an analgesic treatment (Weary et al., 2006). However, some analgesics (e.g. NSAID that inhibit the enzyme cyclooxygenase (COX) and consequently reduce expression of PGE$_2$) will also affect other signs of disease including fever and sickness behaviours. When given before parturition, NSAID may also hinder the normal calving process (Newby et al., 2017)
given the inhibitory effects on prostaglandins. Therefore, when investigating pain and sickness specific behaviours in periparturient cows, other methods of analgesia will need to be explored.

1.3.4 Using behaviours to improve welfare

Sickness behaviour may be a useful early indicator of disease. Early identification of ill animals allows for early treatment, likely increasing the odds of treatment success as has been shown in cows with lameness (Leach et al., 2012). With appropriate medication, early diagnosis and treatment may also allow for timely pain mitigation and reduction of malaise. Commercially available precision dairy technologies now used on farms allow for real-time monitoring of a number of behaviours, such as activity, rumination and feeding time (Borchers and Bewley, 2015; Borchers et al., 2016) and provide the opportunity for routine identification of ill cows. Data collected by automated milking systems may also provide a useful resource to identify ill cows (King et al., 2018).

Another reason that sickness behaviours are worth studying is that this knowledge may be used to inform management of cows that are sick and those prone to become sick. Sickness behaviours are motivated, natural behaviours (Johnson et al., 2002). Allowing animals to perform natural motivated behaviours is an integral part of assuring high animal welfare (Fraser et al., 1997). In fact, not allowing animals to perform sickness behaviours may be detrimental to their recovery; for example, mice that were force-fed had lower chances to survive than mice that were allowed to be anorexic after infection (Murray and Murray, 1979). Thus, increasing our knowledge of which sickness behaviours dairy cows express may serve as basis for future research on environmental preferences of sick cows.
Lastly, measuring (sickness) behaviours may improve our understanding of the effect of treatment on the well-being of cows. If sickness behaviours are associated with a feeling of malaise, then a reduction of sickness behaviours may well indicate a reduction in malaise. Similarly, pain behaviours are frequently being used to assess the effectiveness of analgesic treatment (e.g. Vickers et al., 2005; Chapinal et al., 2010).

1.4 Research questions

The overall aim of my thesis was to broaden our understanding of sickness behaviours in transition dairy cows with inflammatory disease. I first started out with one specific research objective, presented in Chapter 2, which was to determine the change (i.e. reduction) in sickness behaviours after treatment of metritic dairy cows with the NSAID meloxicam. The results of this first study then informed my research objectives of the subsequent studies, presented in Chapters 3 and 4. This first study also generated a comprehensive data set that provided the data I used to address the subsequent objectives.

In Chapter 3, I concentrated on the three days before metritis diagnosis and assessed how primiparous cows changed their standing and lying behaviours at the lying stall. This research was driven by the finding (of the first study) that primiparous cows increased their lying time after NSAID treatment (instead of reducing it as would be expected from a sickness perspective) and by the idea that group-housed ill cows may seek social isolation in the freestalls.

Chapter 4 investigated sickness behaviours expressed by cows with fever, but without other signs of clinical disease. The idea behind this study was to gain a better understanding of sickness behaviours without the influence of localized pain associated with ailments such as metritis or mastitis. I became interested in this question as some of the behavioural changes we
observed in the meloxicam study may have been caused by a reduction of sickness or of pain, suggesting the need of understanding sickness behaviours in more detail.

In summary, I address the following research questions in my thesis:

• Chapter 2: How does providing meloxicam to cows with metritis, in conjunction with antimicrobial treatment, reduce the expression of sickness behaviours, including changes in feeding, social, and lying behaviour?

• Chapter 3: How do primiparous cows diagnosed with metritis differ from healthy cows in stall use, including times spent at the stall, social interactions and lying down related behaviours?

• Chapter 4: How do cows with one or multiple days of fever but without clinical disease differ in feeding, social and lying behaviours from healthy cows without fever?
Chapter 2: Changes in feeding, social, and lying behaviour in dairy cows with metritis following treatment with a non-steroidal anti-inflammatory drug as adjunctive treatment to an antimicrobial


2.1 Introduction

Cows diagnosed with postpartum disease show sickness behaviours, including reduced feed intake, shorter feeding times, changes in social behaviour (Huzzey et al., 2007; Goldhawk et al., 2009), and changes in lying behaviour (Medrano-Galarza et al., 2012; Itle et al., 2015). Metritis – an illness due to uterine infection and characterized by fetid vaginal discharge with or without concurrent fever – is common in dairy cows, with a reported incidence of 10 to 30 %, depending on the intensity and means of detection (Dubuc et al., 2010a; Giuliodori et al., 2013; Pohl et al., 2016). Metritic cows are at risk of reduced milk production and impaired reproduction (Dubuc et al., 2011; Wittrock et al., 2011; Mahnani et al., 2015) and likely experience visceral pain (Stojkov et al., 2015). A wide range of bacteria can result in inflammation of the uterus (Azawi, 2008) with treatment strategies including systemically and locally administered antimicrobials (Haimerl and Heuwieser, 2014).
Only two non-steroidal anti-inflammatory drugs (NSAID), flunixin meglumine and ketoprofen, have been studied in metritic cows. Drillich et al. (2007) treated cows with acute puerperal metritis with antimicrobials (1 mg/kg ceftiofur) either alone or in combination with a single dose of flunixin meglumine (2.2 mg/kg i.v.), and found no beneficial effects of the NSAID on body temperature, reproductive performance, or milk yield. Pohl et al. (2016) found no differences in milk yield and reproductive performance between cows with acute puerperal metritis treated with ketoprofen (3 mg/kg/d for 3 d) or an antimicrobial treatment (1 mg/kg/d ceftiofur for 3 d). However, Amiridis et al. (2001) reported that when metritic cows treated with an antimicrobial and supportive fluid therapy were also provided flunixin meglumine they showed a reduced fever, faster involution of the uterus, and improved reproductive performance.

NSAID primarily act through inhibition of the enzymes cyclooxygenase (COX) I and II. Through synthesis of prostaglandins, COX II promotes a pain and inflammatory response to infection, as well as a range of sickness behaviours (Pecchi et al., 2009), including reduced feed intake, reduced social interactions, and prolonged resting, which are thought to support pathogen elimination by the host (Hart 1988; Dantzer and Kelley, 2007). In contrast, sickness is thought to be associated with a feeling of malaise (Weary et al., 2009). Further, it has also been argued that a sickness response may become mal-adaptive when expressed out of proportion or for a prolonged period (Pecchi et al., 2009). Provision of a NSAID mitigates these sickness behaviours in mice (Crestani et al., 1991; Swiergiel and Dunn, 2001; Soncini et al., 2012), pigs (Johnson and Bore, 1994) and dairy calves (Todd et al., 2010), but it is not clear if provision of a NSAID will reduce sickness behaviours in adult dairy cows.

In contrast to flunixin meglumine and ketoprofen, meloxicam inhibits COX-II more selectively and has a longer elimination half-life (more than 17 h in lactating dairy cows, EMEA
so fewer treatments may be necessary to gain therapeutic benefits. A single dose of meloxicam has been shown to reduce local inflammation (Fitzpatrick et al., 2013) and increase cure, milk production, and reproductive performance (McDougall et al., 2009; 2016) in dairy cows with mastitis. In cows with dystocia meloxicam improved some measures of feeding behaviour (Newby et al., 2013).

This manuscript describes work that was done as part of a larger study that addressed multiple objectives including effects of parity on behaviour (Neave et al., 2017a) and changes in behaviour in the days before diagnosis of metritis (Neave et al., 2017b). The specific objective of this study was to determine if providing meloxicam to cows with metritis, in conjunction with antimicrobial treatment, reduces the expression of sickness behaviours, including changes in feeding, social, and lying behaviour. We hypothesized that cows treated with meloxicam would show reduced sickness behaviours, and that the positive effects of meloxicam would be most pronounced during the first 24 h after treatment when plasma concentrations of the drug are highest.

2.2 Methods

The study was conducted at the UBC Dairy Education and Research Centre in Agassiz, British Columbia, Canada. Animals were cared for following the guidelines of the Canadian Council on Animal Care (2009) and all procedures were approved by the UBC Animal Ethics Committee (Protocols A10-0163 and A14-0040).

2.2.1 Animals, Housing and Diet

From July 2013 to October 2014 all healthy, non-lame cows in the herd were monitored from approximately 21 d before calving to 21 d after calving. In total, health and behaviour data
from 105 primiparous (cows that had never calved prior to enrolment in the study) and 232 multiparous (cows that had at least 1 lactation prior to enrolment in the study) cows were collected by the end of the study. From these animals, cows diagnosed with metritis and no other clinical disease, as described in detail below, were included in the study presented here.

In summary, cows were housed in a prepartum pen beginning 3 wk before their expected calving date and moved to the calving pen when imminent signs of calving were visible (e.g. relaxation of tail ligament, milk let-down). To maintain stocking density at 20 cows in the pen, we moved one cow into the prepartum pen every time one cow was removed. Within 24 h after parturition cows were moved from the calving pen to the postpartum pen. To maintain stocking density at 20 cows this addition coincided with the removal from the pen of the cow with the highest DIM. In both pens, cows had access to 12 electronic feed bins and 2 electronic water bins (Insentec, Marknesse, Holland) and 24 lying stalls equipped with mats (Pasture Mat, Promat Inc., Woodstock, Ontario, Canada) covered with approximately 5 cm of sand. The adjacent maternity pen, a sawdust-bedded pack, housed at most 2 cows at a time and was equipped with 1 Insentec water bin and 6 Insentec feed bins; the number of feed bins filled with feed equaled the number of cows in the pen.

Cows in all pens had ad libitum access to a TMR mixed according to NRC (2001) requirements and fed twice daily at 0700 and 1530 h. Details on feed composition are reported in Neave et al. (2017a). After calving, cows were milked twice daily at approximately 0700 and 1700 h.
2.2.2 Diagnosis of Disease

From 3 until 21 DIM cows were examined for metritis every third day following morning milking. Vaginal discharge was collected manually with a gloved hand and scored on a 5-point scale as described by Huzzey et al. (2007); briefly, 0 = clear mucus, no smell; 1 = mucus with flecks of pus, cloudy or bloody mucus, no smell; 2 = mucus with <50% pus and foul smell; 3 = mucus with >50% pus and foul smell; 4 = watery, brownish discharge with foul smell. Cows that were scored 0 or 1 on all days were categorized as healthy (non-metritic). The first day that the score was 2 or higher the cow was diagnosed with metritis. Body temperature was not part of the case definition.

During each of the seven examinations a blood sample was taken from the coccygeal vein. Blood samples were stored at room temperature for approximately 60 min to allow for clotting before centrifugation (2,800 × g for 15 min at 4°C) and collection of serum. Serum samples were stored at -20°C and analyzed at the University of Guelph Animal Health Laboratory (Guelph, Ontario, Canada) upon completion of data collection. Samples taken from cows on DIM 3, 6 and 12 were analyzed for BHB using Randox BHBA kits (Randox Laboratories Canada Ltd., Mississauga, ON, Canada).

The health of all cows was monitored throughout the study period allowing us to exclude animals affected with clinical diseases other than metritis. Clinical diseases other than metritis, including clinical ketosis, mastitis, milk fever or displacement of the abomasum were diagnosed according to farm protocol by experienced farm staff or the herd veterinarian. Fetal membranes were classified as retained when still attached 24 h after calving. The gait of each cow was assessed weekly in a designated alley with clean, non-slippery concrete flooring. A numerical
scoring system (Flower and Weary, 2005) was used and cows with a score greater than 3 were classified as clinically lame.

2.2.3 **Treatment of Cows with Metritis**

Cows diagnosed with metritis based on their vaginal discharge score up to and including 15 DIM were randomly assigned to receive a single subcutaneous (s.c.) injection of either meloxicam (0.5 mg/kg; Metacam® 20 mg/ml solution, Boehringer Ingelheim GmbH, Ingelheim am Rhein, Germany) or a placebo solution (Boehringer Ingelheim GmbH, Ingelheim am Rhein, Germany), with researchers blind to treatment. Upon diagnosis of metritis, rectal temperature was measured using an electronic thermometer (Nexcare™ Rapid Digital Thermometer, 3M, St. Paul, MN) inserted ~ 10 cm and ensuring contact with the rectal wall, and cows were weighed to determine treatment volume. Researchers were provided two batches of vials, one for primiparous and the other for multiparous cows. All vials (containing either meloxicam or a placebo solution consisting of only the vehicle) were visually identical, and marked only with consecutive numbers. The randomization within each batch was such that within every 4 consecutive vials the meloxicam and placebo treatments were balanced. Within each batch, vials were used in ascending order. Following completion of the study researchers were informed which animals were in the same treatment group (revealed as Group ‘A’ and ‘B’), but remained blind to treatment until completion of the data analyses.

All cows diagnosed with metritis received ceftiofur (2.2 mg/kg BW s.c.; Excenel® RTU sterile suspension, 50 mg/ml as ceftiofur hydrochloride, Zoetis, New Jersey) for 5 consecutive days, injected once daily after the morning milking beginning on the day of diagnosis. Milk of all
enrolled cows was discarded for 96 h following treatment in accordance with the meloxicam label.

### 2.2.4 Sickness Behaviour Measures

**DMI and feeding behaviours.** Intake and feeding behaviours were recorded using the Insentec feeding system. Each cow was electronically recognized by an ear transponder and was electronically identified upon entry to, and exit from, a feed or water bin. All cows had access to all bins in the pen, and a new visit was recorded each time a cow entered a feed bin. The amount of feed consumed (calculated as weight difference from entering to exiting the bin), and the total duration of each visit was recorded by the system (see Chapinal et al., 2007). Behavioural measures derived from these data included daily DMI (kg) and feeding time (min). Feeding rate was defined as g DM consumed per min. A new meal was recorded when there was an interval of at least 20.1 min between two consecutive visits to the feeder (Proudfoot et al., 2009b).

**Social behaviours.** Feed bin replacements were recorded as a measure of social behaviour. A replacement event was automatically recorded using the algorithm validated by Huzzey et al. (2014). Briefly, a replacement was recorded when one cow (actor) entered the bin after another cow (reactor) left the same bin within 26 sec (Huzzey et al., 2014) as measured electronically, typically indicating that the actor used physical contact to force the reactor to leave the feed bin. We report separately the number of events when the focal cow was the actor and reactor in a replacement.

**Lying behaviours.** Electronic data loggers (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA) were attached to the cows’ hind legs using an elastic bandage (Co-Flex, Andover Coated Products Inc., Salisbury, MA) and changed weekly.
G-forces of the y- and z-axes were recorded in 1-min intervals and integrated into an algorithm in SAS (SAS Inc, Cary, NC) developed by the UBC Animal Welfare Program (2013), using the cut-off established by Ledgerwood et al. (2010; see Neave et al., 2017b for further details on logger handling and analysis). Results were summarized into daily lying time (min), number of daily lying bouts (no./d) and average lying bout duration (min).

2.2.5 Study animals

A total of 97 cows diagnosed with metritis were enrolled; 51 multiparous (n = 26 meloxicam, n = 25 placebo) and 46 primiparous (n = 23 meloxicam, n = 23 placebo). Of those, 9 multiparous cows (n = 3 meloxicam, n = 6 placebo) were excluded from analyses due to other health disorders that were diagnosed and treated before or concurrent with metritis treatment (3 cases of clinical ketosis; 1 case of displaced abomasum; 1 case of downer cow syndrome; 4 cases of low feed intake requiring additional supportive measures). Cows that were not clinically diagnosed with ketosis but had serum BHB > 1.2 mmol/L (LeBlanc et al., 2010) (n = 13; average BHB = 2.27 ± 1.13 mmol/L (mean ± SD), range 1.24 to 4.41 mmol/L) were included in the analyses.

From the remaining animals with metritis, two multiparous cows (n = 1 meloxicam, n = 1 placebo) were dropped due to feeding and lying behaviour data losses on d 0. An additional 4 primiparous cows (n = 3 meloxicam, n = 1 placebo) were removed from the lying behaviour data due to malfunctioning Hobo loggers. Thus, the final data set consisted of 41 multiparous cows (n = 23 meloxicam, n = 18 placebo) and all 46 primiparous cows (n = 23 meloxicam, n = 23 placebo) for analyses of feeding and social behaviours, and of 41 multiparous cows (n = 22 meloxicam, n = 19 placebo) and 42 primiparous cows (n = 20 meloxicam, n = 22 placebo) for
lying behaviour analyses. On the morning of treatment, the average body temperature was 39.2°C (± 0.07; mean ± SE) for cows in the meloxicam group and 39.1°C (± 0.07) for cows in the placebo group, of which 30.4 % and 24.4 % had a body temperature of ≥ 39.5 C, respectively. Details on the distribution of DIM of treatment and metritis severity across treatment are displayed in Figure 2.1.

![Figure 2.1 Treatment, DIM of diagnosis and metritis severity of study cows.](image)

**Figure 2.1** Treatment, DIM of diagnosis and metritis severity of study cows.

Distribution of 88 cows (n = 47 meloxicam, n = 41 placebo) across treatment (TRT), DIM of diagnosis and severity of metritis at time of enrollment. Metritis score 2 and score 3 were collapsed into one group (score 3). One multiparous cow (treatment: meloxicam, DIM of diagnosis: d 15, metritis score: 3) was excluded from feeding and social behaviour analyses. One multiparous cow (placebo, d 9, score 4) and 4 primiparous cows (1 cow: meloxicam, d 6, score 3; 1 cow: meloxicam, d 6, score 4; 1 cow: meloxicam, d 9, score 4; 1 cow: placebo, d 12, score 3) were excluded from lying behaviour analyses due to data loss.

### 2.2.6 Statistical Analyses

Before starting the experiment, we determined sample size based on two of the primary feeding behaviour outcomes: DMI and feeding time. The differences in means and standard
deviations of healthy and metritic animals reported by Huzzey et al. (2007) served as the basis for the power analysis. The power analysis indicated that 45 cows per treatment group would be required for detecting treatment differences in DMI (1.7 kg/d, SD = 2.9) and feeding time (25 min/d, SD = 40) with 80 % power and an α = 0.05. We targeted a group size of 50 cows per treatment to allow for some deviation in these variances.

All statistical procedures were performed with SAS (Version 9.4; SAS Institute Inc., Cary, NC). Data were summarized to provide one observation for each behaviour measured per day per cow. Day was corrected to begin at 1000 and end at 1000 h the following day to align with time of treatment (always between 0800 and 1000 h). The day before treatment was defined as d -1 and included the full 24 h up to 1000 h on d 0 (i.e. the time and day of treatment). Residuals from models were used to verify normality and homogeneity of variances. The significance level was set at $P < 0.05$, and tendencies are reported when $P \leq 0.1$.

PROC SUMMARY was used to obtain means and SE for all behaviours on d -1, d 0 and the 5 d after treatment, separately by treatment. We compared differences in baseline (pre-diagnosis) values of the two treatment groups using a t-test. Responses to treatment were analyzed as within-cow changes in behaviour from the day before treatment. Given that meloxicam has an elimination half-life of > 17 h (EMEA 2009), we predicted that the behavioural effects would be greatest during the 24 h period after treatment. Therefore, we ran two separate mixed linear regression models (PROC MIXED in SAS): one examining treatment differences on d 0, and the second for differences on the following days (across d 1 to d 5, accounting for repeated measures). In both cases cow was the experimental unit. The model for the behavioural change on the day of treatment (d 0) contained the following covariates: parity (2 levels: primiparous or multiparous), severity of metritis (2 levels: score 2 or 3, or score 4 on the
day of diagnosis; see Neave et al., 2017b), treatment (2 levels: meloxicam or placebo), DIM at diagnosis (i.e. day of placebo or meloxicam treatment), body temperature at time of treatment, and the BHB concentration measured for each cow on the day of treatment (when diagnosed on d 3, 6 and 12) or on the 3 d before treatment (when diagnosed on d 9 and 15). BW was not included in the model, given its correlation with parity. Milk yield during the first 21 DIM and calving ease were initially included in the model, but neither was significant ($P > 0.1$) and were therefore dropped from the final model. Similarly, all biologically relevant interactions with treatment (parity, metritis severity, and DIM at diagnosis) were tested, but dropped when $P > 0.1$. There were no parity x treatment interactions except for lying time ($P < 0.01$); for this measure the analysis was performed separately for primiparous and multiparous cows.

The model for the change in behaviour from baseline to d 1 to d 5 contained the same explanatory variables and interaction terms, as well as the day relative to treatment (d 1 to d 5) and the interaction of treatment with day relative to treatment (if $P \leq 0.1$). Day relative to treatment was defined as a repeated measure, with cow treated as random effect and specified as subject. The covariance structure was auto-regressive. Consistent with the analysis for d 0, there was an interaction of parity and treatment ($P = 0.02$) for lying time changes, so treatment differences were tested separately by parity.

Results for d 0 are presented as least squares means and SE. To obtain least squares means and SE for d 1 to d 5, day relative to treatment was set as categorical variable and the interaction of treatment with day relative to treatment was retained in the model.
2.3 Results

2.3.1 Behaviour before treatment

Baseline values (d -1) for all feeding and lying behaviours are summarized in Table 2.1.

Table 2.1 Baseline values of feeding, social and lying behaviours.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Meloxicam</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding behaviour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>15.0 ± 0.6</td>
<td>14.4 ± 0.51</td>
</tr>
<tr>
<td>Feeding time (min/d)</td>
<td>155.5 ± 7.59</td>
<td>155.2 ± 6.46</td>
</tr>
<tr>
<td>Visits to feeder (no./d)</td>
<td>50.7 ± 3.01</td>
<td>50.3 ± 3.03</td>
</tr>
<tr>
<td>Meals (no./d)</td>
<td>7.4 ± 0.29**</td>
<td>8.3 ± 0.29**</td>
</tr>
<tr>
<td>Feeding rate (g of DM/min)</td>
<td>105.5 ± 3.55</td>
<td>102.2 ± 3.23</td>
</tr>
<tr>
<td>Social behaviour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actor replacements (no./d)</td>
<td>10.3 ± 0.9</td>
<td>9.3 ± 0.94</td>
</tr>
<tr>
<td>Reactor replacements (no./d)</td>
<td>11.4 ± 0.92</td>
<td>10.9 ± 1.04</td>
</tr>
<tr>
<td>Lying behaviour (min/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>560 ± 26.4</td>
<td>525 ± 26.7</td>
</tr>
<tr>
<td>Multiparous</td>
<td>656 ± 29.4 **</td>
<td>550 ± 40.9 **</td>
</tr>
<tr>
<td>Lying bouts (no./d)</td>
<td>10.8 ± 0.66</td>
<td>11.4 ± 0.74</td>
</tr>
<tr>
<td>Lying bout duration (min/bout)</td>
<td>64 ± 4.7</td>
<td>56 ± 3.5</td>
</tr>
</tbody>
</table>

Baseline values (LS means ± SE) of feeding and social (n = 87 cows; n = 46 meloxicam, n = 41 placebo) as well as lying behaviours (n = 83 cows; n = 42 meloxicam, n = 41 placebo). The meloxicam group contained 23 primiparous and 23 multiparous cows and the placebo group contained 23 primiparous and 18 multiparous cows. The values for feeding, social and lying behaviour are for the 24 h before treatment, but are shown separately for cows later assigned to treatment with meloxicam or a placebo. Values for lying time are reported separately for primiparous and multiparous cows, as there was a treatment × parity interaction for this measure.
Treatments differed \((P = 0.03)\) in number of meals on d-1 with cows in the meloxicam treatment having on average 1 less meal in the 24 h before treatment than cows entering the placebo group. Multiparous (but not primiparous) animals treated with meloxicam also had longer lying times than the placebo treated animals \((P = 0.04)\) on the day before treatment.

2.3.2 Behavioural changes during the 24 h after treatment

DMI and Feeding Behaviours. Cows with metritis treated with meloxicam increased DMI less than did cows in the placebo group \((F_{1,80} = 3.81, P = 0.05\); Figure 2.2, Table 2.2\). Meloxicam-treated cows and placebo-treated cows changed feeding time to a similar extent. However, for the change in time spent at the feed bunk we observed a tendency for an interaction of treatment with parity \((F_{1,79} = 3.07, P = 0.08\), with multiparous cows in the placebo group showing a more pronounced increase in feeding time than those of the meloxicam group, while primiparous cows of both treatment groups increased feeding time to a similar extent. We also observed an interaction of treatment with DIM at diagnosis \((F_{1,79} = 5.04, P = 0.03\); cows treated with meloxicam showed greater increases in feeding time when treated early after calving, but cows that received the placebo showed similar increases in feeding time regardless of DIM.

Cows treated with meloxicam showed a greater increase in the number of visits to the feeder compared with cows that received the placebo \((F_{1,79} = 5.01, P = 0.03\). Again, there was a tendency for an interaction with DIM at diagnosis \((F_{1,79} = 2.89, P = 0.09\); Figure 2.2, Table 2.2\), with treatment differences most apparent for cows diagnosed on d 3, 6 or 9, as meloxicam-treated cows had a decreasing response with increasing DIM, and placebo-treated cows remaining constant across DIM in their increase of feeder visits. Cows treated with meloxicam showed an increase in number of meals while cows that received the placebo decreased their
number of meals \((F_{1,79} = 4.08, P = 0.05)\). There was a tendency for an interaction with DIM at diagnosis \((F_{1,79} = 2.97, P = 0.09)\), where cows treated with meloxicam showed greater increases in meals when treated early after parturition, but placebo-treated cows had similar changes in the number in meals regardless of DIM. There were no treatment effects on feeding rate.

*Social Behaviours.* We observed a tendency for an interaction between treatment and parity for the number of actor replacements \((F_{1,79} = 3.06, P = 0.08)\), where meloxicam-treated primiparous cows increased their actor replacements more, but meloxicam-treated multiparous cows increased their actor replacements less than placebo cows of the same parity group. We found no treatment effect for the number of reactor replacements.

*Lying Behaviours.* Multiparous cows treated with meloxicam reduced lying time compared with multiparous cows that received the placebo \((F_{1,35} = 5.03, P = 0.03; \text{Table } 2.2)\), but there was no treatment effect for primiparous cows. Cows treated with meloxicam tended to show a lesser increase in the number of lying bouts compared with the cows that received the placebo \((F_{1,77} = 3.63, P = 0.06; \text{Table } 2.2)\). There was no treatment difference for the change in lying bout duration.
Table 2.2 Behaviour changes in the 24 h after treatment.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Meloxicam</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feeding behaviours</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>1.8 ± 0.61</td>
<td>3.3 ± 0.60</td>
<td>0.05</td>
</tr>
<tr>
<td>Feeding time (min/d)</td>
<td>15.8 ± 5.43</td>
<td>31.7 ± 5.79</td>
<td>0.27</td>
</tr>
<tr>
<td>Visits to feeder (no./d)</td>
<td>9.6 ± 2.44</td>
<td>4.4 ± 2.39</td>
<td>0.03</td>
</tr>
<tr>
<td>Meals (no./d)</td>
<td>0.2 ± 0.31</td>
<td>-0.2 ± 0.31</td>
<td>0.05</td>
</tr>
<tr>
<td>Feeding rate (g of DM/min)</td>
<td>-0.9 ± 2.62</td>
<td>-2.0 ± 2.56</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Social behaviours</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actor Replacements (no./d)</td>
<td>2.3 ± 1.12</td>
<td>1.4 ± 1.1</td>
<td>0.55</td>
</tr>
<tr>
<td>Reactor Replacements (no./d)</td>
<td>2.1 ± 1.03</td>
<td>1.6 ± 1.01</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Lying behaviours</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lying time (min/d) ¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>48 ± 21.3</td>
<td>5 ± 21.7</td>
<td>0.13</td>
</tr>
<tr>
<td>Multiparous</td>
<td>-27 ± 28.0</td>
<td>50 ± 25.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Lying bouts (no./d)</td>
<td>-0.1 ± 0.54</td>
<td>1.2 ± 0.52</td>
<td>0.06</td>
</tr>
<tr>
<td>Lying bout duration (min/bout)</td>
<td>0.5 ± 3.3</td>
<td>-2.6 ± 3.2</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Changes in feeding behaviour (DMI, feeding time, visits, meals and feeding rate), social behaviour (actor and reactor replacements) and lying behaviour (lying time, lying bouts, lying bout duration) in the 24 h following treatment compared to the day before treatment (LS means ± SE). Feeding and social behaviours were analyzed for 87 cows (n = 46 meloxicam, n = 41 placebo) and lying behaviours were analyzed for 83 cows (n = 42 meloxicam, n = 41 placebo).

¹ P < 0.01 for treatment x parity interaction, therefore lying times were analyzed separately for primiparous and multiparous cows.
Figure 2.2 Feeding behaviours on the day before and the day of treatment.
Figure 2.2: DMI (A), feeding time (B) and visits to the feeder (C) measured for each cow (n = 46 meloxicam, n = 41 placebo) on the day before treatment (d -1; black dot) and on the day of treatment (d 0; grey dot). The difference between both dots represents the within-cow change from d-1 to d 0 which was the dependent variable in our analysis. For each graph and behaviour, cows were sorted by increasing baseline (d -1) value within their treatment group; therefore, consecutive numbers of cows do not correspond to a particular cow and across graphs, but are merely displayed for visualization purposes.

2.3.3 Behaviour changes from the day before treatment to d 1 to 5 after treatment

Over d 1 to 5, meloxicam-treated cows increased their number of daily meals while cows in the placebo group decreased their number of meals compared to the day before treatment ($F_{1,80} = 7.55, P < 0.01$; Table 2.3). Multiparous cows treated with meloxicam decreased their lying times, while those in the placebo group increased their lying times ($F_{1,33} = 7.32, P = 0.01$). There was, however, an interaction between treatment and DIM at diagnosis ($P = 0.02$), and a tendency for an interaction with metritis severity ($P = 0.08$). Meloxicam-treated cows increased lying times to a greater extent with increasing DIM at diagnosis (with the exception of cows diagnosed on 15 DIM where cows decreased lying times), but placebo-treated cows changed lying time such that cows diagnosed early after calving increased lying times, but cows diagnosed later after calving decreased lying times. Further, cows with metritis score 3 increased lying times and cows with metritis score 4 decreased lying times; the increase in lying times for cows with score 3 was more pronounced in meloxicam-treated cows, but the decrease in lying times for cows with score 4 was similar in both treatment groups.

Primiparous cows treated with meloxicam increased their lying times more than placebo-treated primiparous cows ($F_{1,38} = 4.81, P = 0.03$), but there was no such difference for multiparous cows.
## Table 2.3 Behaviour changes on d 1 to 5 after treatment.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Treatment</th>
<th>d 1</th>
<th>d 2</th>
<th>d 3</th>
<th>d 4</th>
<th>d 5</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding behaviours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>Meloxicam</td>
<td>2.3 ± 0.6</td>
<td>2.3 ± 0.6</td>
<td>2.1 ± 0.6</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.6</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2.2 ± 0.6</td>
<td>2.5 ± 0.6</td>
<td>2.4 ± 0.6</td>
<td>2.3 ± 0.6</td>
<td>2.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Feeding time (min/d)</td>
<td>Meloxicam</td>
<td>27.2 ± 7.4</td>
<td>33.5 ± 7.4</td>
<td>36.4 ± 7.4</td>
<td>43.7 ± 7.4</td>
<td>33.7 ± 7.4</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>27.3 ± 7.5</td>
<td>35.5 ± 7.5</td>
<td>34.1 ± 7.6</td>
<td>34.0 ± 7.6</td>
<td>36.6 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>Visits (no./d)</td>
<td>Meloxicam</td>
<td>9.2 ± 2.7</td>
<td>10.2 ± 2.7</td>
<td>11.6 ± 2.7</td>
<td>13.5 ± 2.7</td>
<td>14.5 ± 2.7</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>6.1 ± 2.8</td>
<td>8.7 ± 2.8</td>
<td>8.0 ± 2.8</td>
<td>9.0 ± 2.8</td>
<td>9.5 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Meals (no./d)</td>
<td>Meloxicam</td>
<td>0.4 ± 0.3</td>
<td>0.0 ± 0.3</td>
<td>-0.2 ± 0.3</td>
<td>0.2 ± 0.3</td>
<td>-0.1 ± 0.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>-0.5 ± 0.4</td>
<td>-0.8 ± 0.4</td>
<td>-1.0 ± 0.4</td>
<td>-0.8 ± 0.4</td>
<td>-1.0 ± 0.4</td>
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</tr>
<tr>
<td>Feeding rate (g of DM/min)</td>
<td>Meloxicam</td>
<td>-3.5 ± 2.9</td>
<td>-7.9 ± 2.9</td>
<td>-8.7 ± 2.9</td>
<td>-9.4 ± 2.9</td>
<td>-5.7 ± 2.9</td>
<td>0.53</td>
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<tr>
<td></td>
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<td>-5.3 ± 2.9</td>
<td>-7.7 ± 2.9</td>
<td>-7.2 ± 2.9</td>
<td>-7.2 ± 3.0</td>
<td>-10.0 ± 3.0</td>
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<tr>
<td>Behaviour</td>
<td>Treatment</td>
<td>d 1</td>
<td>d 2</td>
<td>d 3</td>
<td>d 4</td>
<td>d 5</td>
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<tr>
<td>Social behaviours</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Actor replacements (no./d)</td>
<td>Meloxicam</td>
<td>3.4 ± 1.1</td>
<td>2.8 ± 1.1</td>
<td>4.2 ± 1.1</td>
<td>3.9 ± 1.0</td>
<td>4.3 ± 1.0</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2.7 ± 1.1</td>
<td>2.7 ± 1.1</td>
<td>3.9 ± 1.1</td>
<td>4.8 ± 1.1</td>
<td>3.2 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Reactor replacements (no./d)</td>
<td>Meloxicam</td>
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<td>3.0 ± 1.0</td>
<td>3.8 ± 1.0</td>
<td>3.7 ± 1.0</td>
<td>4.8 ± 1.0</td>
<td>0.33</td>
</tr>
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<td>Placebo</td>
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<td>3.3 ± 1.1</td>
<td>3.1 ± 1.1</td>
<td>4.3 ± 1.1</td>
<td>2.8 ± 1.1</td>
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<td>Lying behaviours</td>
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<tr>
<td>Lying time (min/d)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>Meloxicam</td>
<td>50 ± 21.3</td>
<td>43 ± 21.7</td>
<td>29 ± 21.8</td>
<td>39 ± 21.8</td>
<td>53 ± 22.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0 ± 21.3</td>
<td>-12 ± 21.3</td>
<td>27 ± 21.6</td>
<td>37 ± 21.7</td>
<td>-14 ± 21.8</td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td>Meloxicam</td>
<td>46 ± 35.2</td>
<td>-14 ± 35.2</td>
<td>-9 ± 35.2</td>
<td>20 ± 35.2</td>
<td>-35 ± 35.2</td>
<td>0.01</td>
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<td></td>
<td>Placebo</td>
<td>71 ± 33.4</td>
<td>68 ± 33.9</td>
<td>46 ± 34.1</td>
<td>88 ± 34.1</td>
<td>27 ± 34.2</td>
<td></td>
</tr>
<tr>
<td>Lying bouts (no./d)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meloxicam</td>
<td>1.3 ± 0.6</td>
<td>1.2 ± 0.6</td>
<td>0.4 ± 0.6</td>
<td>0.4 ± 0.6</td>
<td>0.7 ± 0.6</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.8 ± 0.6</td>
<td>0.8 ± 0.6</td>
<td>1.1 ± 0.6</td>
<td>1.4 ± 0.6</td>
<td>1.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Lying bout duration (min/bout)</td>
<td>Meloxicam</td>
<td>-2.7 ± 4.0</td>
<td>-8.1 ± 4.0</td>
<td>-4.3 ± 4.0</td>
<td>-2.7 ± 4.0</td>
<td>-9.0 ± 4.0</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>-1.5 ± 3.9</td>
<td>-0.4 ± 3.9</td>
<td>-3.4 ± 4.0</td>
<td>-0.5 ± 4.0</td>
<td>-3.1 ± 4.0</td>
<td></td>
</tr>
</tbody>
</table>

Changes in feeding and social (n = 87 cows; n = 46 meloxicam, n = 41 placebo) as well as lying behaviours (n = 83 cows; n = 42 meloxicam, n = 41 placebo) from day before treatment (d -1) to d 1 to d 5 after treatment (LS means ± SE).
2.4 Discussion

Changes in feeding, social and resting behaviours have been associated with sickness across different species (Hart, 1988; Dantzer and Kelley, 2007), including in dairy cows with metritis (e.g. reduction in DMI and feeding time; Hammon et al., 2006; Huzzey et al., 2007). NSAID have the potential to reduce sickness behaviours through inhibition of prostaglandin synthesis (Pecchi et al., 2009). We sought to test if cows with metritis benefit from meloxicam, as measured by reduced sickness behaviours, when given as adjunctive treatment to an antimicrobial. We expected that cows in both treatment groups would show fewer sickness behaviours after treatment with an antimicrobial (e.g. increase of DMI), but that these changes would be of greater magnitude in meloxicam treated cows especially during the first 24 h after treatment.

As summarized in Table 2.4, the results corresponded poorly with our predictions. Both treatment groups improved on d 0 as expected, but there was no clear or consistent additional benefit of meloxicam treatment. These inconsistencies make the results of this study hard to interpret. For example, meloxicam-treated cows showed an increase in number of visits to the feeder relative to the placebo-treated cows, possibly related to an increase in exploratory behaviour. Reductions in exploratory behaviour in rats have been associated with biochemical changes in the brain related to the psychological experience of ‘fatigue’ described in humans (Gaykema and Goehler, 2011), a subjective state associated with malaise (Glaus et al., 1996; Ream and Richardson, 1997). However, we found no effect of the meloxicam treatment on DMI or feeding time (i.e., the increased visits to the feeder did not result in or were a result of greater intake), countering our prediction that treated animals would show increased appetite associated with reduced feelings of sickness-induced malaise.
Table 2.4 Predicted and observed within-animal changes for meloxicam and placebo treated cows

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Prediction</th>
<th>Results (24 h after treatment)</th>
<th>Results (d 1 to d 5 after treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direction of change</td>
<td>Difference between treatment groups</td>
<td>Direction of change</td>
</tr>
<tr>
<td>Feeding behaviours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI</td>
<td>+</td>
<td>M &gt; P</td>
<td>+</td>
</tr>
<tr>
<td>Feeding time</td>
<td>+</td>
<td>M &gt; P</td>
<td>+</td>
</tr>
<tr>
<td>Visits to the feeder</td>
<td>+</td>
<td>M &gt; P</td>
<td>+</td>
</tr>
<tr>
<td>Feeding rate</td>
<td>NA¹</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>No. of meals²</td>
<td>NA¹</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Social behaviours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actor replacements</td>
<td>+</td>
<td>M &gt; P</td>
<td>+</td>
</tr>
<tr>
<td>Reactor replacement</td>
<td>-</td>
<td>M &gt; P</td>
<td>+</td>
</tr>
<tr>
<td>Lying behaviours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lying time⁴,⁵</td>
<td>NA⁶</td>
<td>NA</td>
<td>Primi⁷: +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Multi⁷: -</td>
</tr>
<tr>
<td>No. of lying bouts</td>
<td>NA⁶</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Lying bout duration</td>
<td>NA⁶</td>
<td>NA</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2.4 Predicted and observed within-animal changes for each measured behaviour for meloxicam (M) and placebo (P) treated cows. Predictions are based on the original study proposal from 2013, unless indicated otherwise (NA: not applicable). The direction of change is reported as increase (+) or decrease (-) of within-animal change from baseline (d -1) to observation day after treatment (d 0 to d 5). Given our prediction that behavioural differences between treatment groups would be most pronounced during the first 24 h after treatment, results are reported separately for this period (d 0) and the following days (d 1 to d 5). Greater-than (‘>’) and smaller-than (‘<’) signs indicate significantly greater and smaller within-animal change from baseline for meloxicam treated cows, respectively, and, when parenthesized, a tendency for greater and smaller within-animal change from baseline for meloxicam treated cows, respectively. Equal-sign (‘=’) indicates no difference in within-animal change from baseline between treatment groups.

1 Additional measure added during the study, no predictions in original study proposal
2 Meloxicam treated cows ate approximately 1 meal less on day before treatment
3 Meloxicam treated cows increased and placebo treated cows decreased number of daily meals
4 Lying time: treatment x parity interaction: $P < 0.01$, therefore lying times were analyzed separately for primiparous and multiparous cows
5 Meloxicam treated multiparous cows had longer lying times (approximately 100 min) on day before treatment
6 Prediction in study proposal indicated expected ‘effect’ but not direction of effect of meloxicam on lying behaviours
7 Primi: primiparous; Multi: multiparous
8 Meloxicam treated cows decreased lying time and placebo treated cows increased lying time

Metritis in dairy cows is accompanied by visceral pain (Stojkov et al., 2015). Even though we did not measure pain behaviours specifically, some of the observed behavioural changes, such as increased feeder visits, may also be explained by the analgesic properties of meloxicam. Reduced locomotor behaviour has been described as indicative of pain in rats after abdominal incision and irritation of viscera (Martin et al., 2004), and reduced activity has also been reported in cows diagnosed with metritis (as measured with neck-mounted accelerometers; Liboreiro et al., 2015; Stangaferro et al., 2016b). Moreover, parturition is believed to be painful (Mainau and Manteca, 2011) which may explain the higher activity (more steps taken) of primiparous cows when treated with meloxicam versus a placebo (Mainau et al., 2014).
Increased visits to the feed bunk arguably require a higher level of activity and may therefore reflect increased movement of the treated animals. However, if analgesic effects of meloxicam allowed cows to move with less pain we would have expected these cows to also have a greater increase in lying bouts compared with placebo treated animals; Barrier et al. (2014) reported that beef cows treated with meloxicam immediately before caesarean section, a procedure reported to cause pain despite anesthetic (Kolkman et al., 2010), had a higher number of lying bouts on the day following surgery compared with animals that received a placebo.

Not surprisingly, most differences in behavioural change were only evident in the first 24 h after treatment, likely due to the elimination half-life of meloxicam (EMEA, 2009). However, it remains unclear why meloxicam and placebo-treated cows in the current study differed in some behavioural changes in the days well beyond the elimination half-life (EMEA 2009) and the expected efficacy of meloxicam.

Previous work on rodents (Hennessy et al., 2014) and laying hens (Gregory et al., 2009) has shown that the intensity with which ill animals express sickness behaviours can be influenced by their social environment. The social environment may similarly influence the expression of sickness behaviour in dairy cows and with that the efficacy of meloxicam in reducing such behaviours. Given that healthy primiparous and multiparous cows differ in behaviours during transition when housed together (Kaufmann et al., 2016; Neave et al., 2017a), the social environment may also explain why treatment varied with parity at least for some responses (i.e. feeding time, lying time). Alternatively, the parity effect may be explained by a difference in analgesic efficacy; work in humans (Yeh et al., 2005) demonstrated that the analgesic effect of tenoxicam on uterine cramping following a caesarean section was greater in primiparous women compared to multiparous women.
Work on laboratory rodents found dose-dependent effects of meloxicam on a fever response (i.e. increasing effects with increasing dose; Engelhardt et al., 1995), regulated by similar mechanisms as sickness behaviours (Pecchi et al., 2009). Although meloxicam did not prevent a fever response or a decline in DMI when given to dairy cows at the time of an intramammary LPS infusion, it did shorten the duration of the fever response, reduce pain-specific behaviours and signs of local inflammation compared with animals that did not receive the NSAID (Fitzpatrick et al., 2013). Given that we did not include pain-specific behaviours in our study we are unable to make inferences on the analgesic efficiency of meloxicam at the dose provided; it is possible that higher dosages of meloxicam may have been more effective.

The improvement in most of our measures in the days following diagnosis, irrespective of meloxicam treatment, was likely explained by the effect of ceftiofur, an antimicrobial approved for metritis treatment in dairy cows (Haimerl and Heuwieser, 2014; Reppert, 2015; Haimerl et al., 2017). To our knowledge, no pharmacological studies have investigated interactions between ceftiofur and meloxicam, although the combination of ceftiofur and another NSAID, acetyl salicylate, did not alter the pharmacokinetics of either drug in adult dairy cattle (Whittem et al., 1995, 1996). Little is known about how antimicrobials in general, and ceftiofur in particular, change behaviour in dairy cattle, let alone the time course of these changes. In cows with naturally occurring mastitis, intra-mammary antimicrobial treatment improved feeding behaviours, with changes most pronounced on the second day after treatment (Sepúlveda-Varas et al., 2016). Based on these results, and given that the elimination half-life of meloxicam is > 17 h (EMEA, 2009), we had expected incremental improvements in behaviours within the first 24 h due to the meloxicam. That both the meloxicam and placebo group improved on the day of
treatment suggests that the antimicrobial began to take effect within this first day, as reported for antipyretic effects of ceftiofur (Chenault et al., 2004).

The dosage of ceftiofur was according to North American drug label instructions (2.2 mg/kg BW for 5 d). This is higher than for some European labels (e.g. UK, Germany; 1 mg/kg BW for 5 d). The higher dosage is known to be more effective at reducing body temperature in cows with metritis with fever than the lower dosage (Chenault et al., 2004). Also, there is some evidence that these third generation cephalosporins have analgesic effects by inhibiting a neurotransmitter pathway in the central nervous system (Rothstein et al., 2005; Lin et al., 2011; Stepanovic et al., 2014). These analgesic effects were further fortified by some NSAID in rats and mice (Stepanovic et al., 2014) but no work has shown this in cattle.

Our failure to show a clear benefit to the NSAID as adjunctive treatment is in line with other studies. Recent research investigating a combined treatment of antimicrobials plus a NSAID in beef cattle with bovine respiratory disease found little beneficial effect of the anti-inflammatory drug (Wilson et al., 2015; Toaff-Rosenstein et al., 2016). Given the growing concerns regarding the use of antimicrobials in farm animals (Van Boeckel et al., 2015) the treatment of metritis with antimicrobials may become more scrutinized, especially given the high self-recovery rates for this disease (McLaughlin et al., 2012). One clinical trial (Pohl et al., 2016) suggests that initial therapy of metritis with an NSAID alone may reduce overall antimicrobial use with similar clinical outcomes for the cow. If antimicrobial use is reduced, NSAID may play a greater role and we encourage further studies to investigate performance and welfare outcomes for treatment of metritis with NSAID, antimicrobial, or both.
2.5 Conclusions

A single administration of meloxicam as adjunctive treatment to antimicrobial therapy had no consistent effect on sickness behaviors in cows with metritis. Analgesic effects of meloxicam may have contributed to some of the behavioral changes we observed. The efficiency of meloxicam in mitigating pain associated with metritis needs to be tested more specifically.
Chapter 3: Effects of metritis on stall use and social behaviour at the lying stall


3.1 Introduction

Reduced feeding, decreased social interactions and increased resting are associated with the onset of sickness in many mammals (Dantzer and Kelley, 2007). In dairy cows, especially feeding behaviours and social behaviours at the feed bunk have been explored, and some of these behaviours have shown promise as early indicators of disease. Compared with healthy cows, ill cows consume less DM (Huzzey et al., 2007; Fogsgaard et al., 2015), spend less time feeding (González et al., 2008; Goldhawk et al., 2009), engage in fewer competitive interactions at the feeder (Huzzey et al., 2007) and avoid the feed bunk at peak feeding times when competitive interactions are most common (Sepúlveda-Varas et al., 2016).

Avoidance of social interactions by ill cows may also be evident in contexts other than feeding. For example, cows that became ill with either mastitis or metritis in the days following calving increased use of an area visually separating them from the remainder of the herd (Proudfoot et al., 2014). Freestalls do not provide visual separation, but as partitions at the feed bunk reduce competitive social interactions during feeding (DeVries and von Keyserlingk, 2006; Hetti Arachchige et al., 2014), lying stall partitions that separate animals from one another may also offer a protective environment and therefore may be more attractive when animals are ill.
Dairy cows alter lying behaviour in response to sickness (Ile et al., 2015; Sepúlveda-Varas et al., 2014) and pain (Mølgaard et al., 2012). Multiple studies have reported shorter lying times in cows with clinical mastitis compared with healthy animals, suggesting that cows may be avoiding painful pressure on the inflamed udder (Siivonen et al., 2011; Fogsgaard et al., 2012, 2015). Some earlier work has found that cows experiencing painful conditions (e.g. dystocia and mastitis) lie down more often (Proudfoot et al., 2009a, Boyer des Roches et al., 2017), but other work has shown that cows with metritis lie down less often in the days before diagnosis (Neave et al., 2018). However, changes in lying time in response to disease are not always observed, for instance, cows with metritis, an infectious disease associated with visceral pain (Stojkov et al., 2015), showed no difference in lying times compared with healthy cows (Neave et al., 2018), despite showing other signs of malaise, e.g. reduced feed intake (Huzzey et al., 2007; Schirmann et al., 2016) and reduced activity (measured by neck-mounted accelerometer; Liboreiro et al., 2015). In summary, sickness and pain can both lead to behavioural changes, but these changes may be differ depending upon the cause making it difficult to predict how cows that experience both sickness and pain will respond. For example, metritis is frequently associated with a febrile response (Sheldon et al., 2006) that might be expected to increase lying time as ill animals typically rest for prolonged periods (Hart, 1988); however, metritis is also associated with visceral pain (Stojkov et al., 2015), possibly resulting in avoidance of lying down or hindering the lying down movement.

To our knowledge no research has investigated if, in response to malaise or pain, cows alter their behaviour at the lying stall independently from lying times. Thus, the objective of our study was to investigate stall use, including times spent at the stall, social interactions and lying down related behaviours, by primiparous cows diagnosed with metritis. We hypothesized that
cows with metritis would increase stall usage, specifically standing in the stall, engage in fewer antagonistic interactions and show more aborted lying events in the days prior to diagnosis.

3.2 Methods

This study used data generated by the study presented in Chapter 2 (published: Lomb et al., 2018a). Housing, management and diagnostic criteria and procedures are briefly recounted here and methods pertaining specifically to this study are added where appropriate.

3.2.1 Housing and Management

During the study, behaviours and the health status of all animals in the herd (n = 337 Holstein cows) were monitored from approximately 3 wk before to 3 wk after calving. By the end of the study period, data for 105 primiparous cows had been collected.

Within 24 h after parturition, all cows were moved into a mixed-parity postpartum pen where they remained for a minimum of 21 d. Whenever a freshly calved cow was introduced the cow with the highest DIM (DIM at removal ranged from 21 to 37, with a mean of 30) was removed to maintain a stocking density of 20. On a single occasion in spring 2014, where there was a longer lag between calving events, 8 cows with high DIM were replaced with pregnant filler cows to maintain stocking density. Feed and water were provided from 12 feed bins and 2 electronic bins, respectively (INSENTEC, Marknesse, Holland; validated by Chapinal et al., 2007). The lying area consisted of 24 lying stalls, equipped with mats (Pasture Mat, Promat Inc., Woodstock, Ontario, Canada) covered with 5 cm of sand. The stalls were 120 cm wide (center to center) and 260 cm long, with the brisket board placed 170 cm from the inside of the back curb. The neck rail was positioned at 115 cm height from the bedded surface, 165 cm as measured from the horizontal axis, and 200 cm as measured from the diagonal axis to the inside of the back curb.
curb. Stalls were raked clean twice daily when the cows were brought up for milking, and fresh sand was added on Monday and Friday every week of the study. Concrete floors in both alleys (i.e. feed alley and the alley between lying stalls) and cross-over alleys were covered with vulcanized rubber mats (Gummiwerk KRAIBURG Elastik, Tittmoning, Germany).

Cows were milked twice daily at approximately 0700 h and 1700 h. At approximately 0800 h and 1600 h, cows were provided fresh feed formulated to meet the NRC (2001) nutrient requirements. Feed samples were taken once weekly, pooled into monthly samples and analyzed. The post-partum cow TMR included 26% corn silage, 13% grass silage, 7% alfalfa hay, 4% grass hay and 50% grain concentrate mash (DM: 50.35 ± 2.5%, CP: 18.3 ± 0.58% DM, ADF: 18.1 ± 0.71% DM, NDF: 28.5 ± 1.2% DM, and NE₇₇: 1.72 ± 0.014 Mcal/kg; described in full by Neave et al., 2017).

3.2.2 Diagnosis of disease

Based on the methodology described by Huzzey et al. (2007), cows were screened for metritis on every third day after calving, beginning at 3 DIM and lasting until 21 DIM. For this purpose, animals were moved into a sorting area immediately after morning milking (between 0800 and 1000 h) and restrained using head locks. Metritis diagnosis was based on the visual appearance (color, consistency, presence of pus) and olfactory sensation (no odor/foul smell) of vaginal discharge, using a 5-point scoring system from 0 to 4 (described by Huzzey et al. 2007): cows with a clear, cloudy or bloody mucus and no smell were diagnosed with a healthy uterus (score 0 and 1), while cows with foul smelling discharge that either contained pus or was of watery, brownish character were diagnosed with metritis (scores 2 – 4). On the morning of diagnosis (between 0800 and 1000 h), cows with metritis were enrolled in the study described by
Lomb et al. (2018a; presented in Chapter 2) where cows were randomly treated with the non-steroidal anti-inflammatory drug meloxicam or a placebo, in addition to an antimicrobial.

Retained placenta was diagnosed when fetal membranes were still attached 24 h after calving. Other clinical diseases (i.e. mastitis, ketosis, displacement of abomasum) were diagnosed based on standard farm protocols or by the herd veterinarian. Calving ease was categorized as unassisted, easy pull (i.e. 1 person assisted) or dystocia (i.e. hard pull with more than 1 person assisting or malpresentation of the calf). Beginning the first day after calving, the rectal body temperature of each animal was taken daily between 0800 h and 1200 h, using a digital thermometer (Nexcare™ Rapid Digital Thermometer, 3M, St. Paul, MN) that was inserted 10 cm into the rectum and pressed against the lateral wall. All cows were weighed twice within the first 3 d after calving, with values averaged to provide a mean BW. Cows were categorized as healthy if they were not diagnosed with any clinical disease, including dystocia, and did not have a body temperature > 39.5°C more than once during the 21 d observation period.

### 3.2.3 Study animals

All primiparous cows diagnosed with a metritis score 4 were considered for participation in the study. Out of the 26 initially eligible cows 10 were excluded due to incomplete video records of the 3 days before metritis diagnosis. The remaining 16 cows were diagnosed with metritis on either DIM 6 (n = 11), 9 (n = 3) or 12 (n = 2), had an average BW of 574 ± 65.3 kg and an average daily milk yield over the first 21 DIM postpartum of 24 ± 4.9 kg/d.

From the healthy primiparous cows (n = 42), 16 animals were selected as match pairs to the metritic animals. The identification of the match pairs took place by firstly, excluding any
animals with missing video data (n = 12). Secondly, of the remaining animals, healthy cows were matched with metritic cows based on BW, and, when possible calving date. Lastly, when there was more than 1 cow with a similar BW and calving date, selection was random. The identified 16 healthy cows had an average BW of 603 ± 51.6 kg and an average daily milk yield over the first 21 DIM of 24 ± 4.2 kg/d. With the exception of one healthy cow that had a metritis score of 0, the remaining 15 healthy animals all had a metritis score of 1.

3.2.4 Behavioural measures

All behaviours were assessed from video. Four cameras (CCTV camera, model WVCW504SP, Panasonic, Osaka, Japan) were mounted 6 m above the experimental pen, 2 above the feed alley and 2 above the lying stalls. Cameras were connected to a digital video surveillance system (GeoVision, GeoVision Inc., Corona, CA). Cows were marked on their back and sides with alphanumerical symbols using hair dye to allow for individual identification.

Behaviour of metritic cows were assessed on the 3 d before diagnosis (d -3 to d -1), before any treatment was given to the participating cows (Lomb et al., 2018a; presented in Chapter 2). The 3 d window for behavioural observations was chosen given that cows were scored as clinically healthy (non metritic) during the health check 3 d before metritis diagnosis. For healthy animals, the 3 corresponding DIM to the metritic pair were analyzed. To account for the time of diagnosis, days were adjusted to start at 1000 h; for example, d -1 included the 24 h interval from 1000 h on the day before diagnosis to 1000 h on the day of diagnosis.

Behaviours were scored by 3 trained observers, and agreement was tested using Cohen’s kappa (Cohen, 1960). Observers reached excellent inter- and intra-observer agreement (κ > 0.75;
Kaufman and Rosenthal, 2009) for all reported behaviours. One of these observers, blind to health status of the animal, scored all aborted lying events.

*Position and time spent in the stall.* The overall time each cow spent at the lying stall, as well as the positions she adopted in the stall, was measured using 5-min scan samples. The specific positions recorded were: *Lying* -- abdomen touching the ground; *Perching* -- two front feet in the stall; and, *Standing fully in stall* -- all four feet in the stall. If at the moment of the scan the cow was changing her position, the position she adopted at the end of the movement was recorded.

The duration of each behaviour in min/d was estimated by multiplication of number of scans by 5 (the length of the sampling interval, described and validated for standing behaviour in cattle by Chen et al., 2016). Reported are lying time (min/d), time spent perching (min/d), time standing fully in the stall (min/d), time spent standing in the stall (min/d), and total time at the stall (min/d). The total time standing in the stall was defined as the sum of time spent perching and standing fully in the stall. The total time at the stall was defined as the sum of lying and standing time in either position in the stall.

*Social behaviour.* Social behaviours at the lying stalls were observed by continuous assessment of the video. Social interactions at lying stalls included: *Actor replacement* -- the focal cow used physical contact (e.g. head butt, push) to force another cow out of the stall, and thereafter either entered the stall or remained outside the stall; and *Reactor replacement* -- the focal cow was forced out of the stall by physical contact (e.g. head butt, push) from another cow. The position of the reactor cow (lying, perching or standing fully in the stall) at the time of displacement was also noted for each event. The number of times she was replaced was
calculated in relation to the time she spent in that position (i.e. number of position
replacements/h spent in this position).

Lying down associated behaviours. Continuous observations were also used to assess
behaviours related to lying down. To lie down in the stall, a cow must enter with all four feet. The
cow can then perform one of the following behaviours:

Visit -- the cow leaves without lying down.

Aborted lying events -- the cow displays behaviours associated with lying down but does
not do so. This was recorded when at least one of the following behaviours was observed: 1) while
lowering her head to the ground the cow completed at least 1 sweeping head movement (Österman
and Redbo, 2001) that crossed her centerline (Tucker and Weary, 2004), or 2) while her head was
lowered to the ground, she completed a minimum of 2 steps or leg movements towards the center
of her body (underneath), or 3) the cow lowers herself from a standing position to her carpal joints
but then moves back into the standing position without lying down.

When cows actually completed the lying down movement, the latency to lie down was
recorded as the time from the first aborted lying event until she successfully lay down; intervals
that precluded stall use (e.g. milking, sand delivery) were not included. We report the daily number
of visits to the lying stalls, the daily number of aborted lying events and the average latency to lie
down (min/lying bout).

3.2.5 Statistical analyses

All statistical analyses were performed using SAS (Version 9.4; SAS Institute Inc., Cary,
NC), with cow as experimental unit and behaviours summarized per cow and day. Variables
were screened by health group using PROC UNIVARIATE. Four variables (standing fully in the
stall, reactor replacements when standing fully in the stall, aborted lying events and average latency to lie down) were transformed using the $\log_{10}$ transformation to normalize distributions for further analyses.

Behavioural differences between healthy and metritic cows were analyzed using PROC MIXED in SAS. The initial model included BW (kg), DIM at diagnosis (diagnosis day; 3 levels: 6, 9 and 12) and health status (2 levels: healthy vs. metritis) as between cow factors, and day relative to diagnosis (observation day; 3 levels: d -3, d -2 and d -1) as a within cow factor. The interaction of health status with observation day was also offered to the model; this interaction was evident (with $P \leq 0.10$) for two of the outcome variables (standing fully in the stall and reactor replacements). Hence, all analyses were performed by observation day, with BW, diagnosis day and health status as covariates in the model.

Results for normally distributed variables are presented as LS means and SE. Results for variables that were $\log_{10}$ transformed are reported as back-transformed geometric means and 95% CI on the original scale. The significance level was set at $P < 0.05$ and tendencies are reported when $P \leq 0.10$.

We observed a low frequency of replacements out of a lying position (metritis: 0 (0 – 0.33); healthy: 0.17 (0-0.33) no./60 min; median (Q1 – Q3)). Therefore, treatment differences were not tested for this measure. Differences in lying time and daily number of lying bouts were previously reported by Neave et al. (2018) where cows with metritis had fewer, but longer lying bouts; these measures are only reported here when necessary to supplement outcome measures of interest.
3.3 Results

On d -2, cows diagnosed with metritis tended to spend more time standing in the stall than healthy cows ($F_{1,28} = 3.62, P = 0.07$); the difference was more pronounced on d -1 ($F_{1,28} = 8.92, P < 0.01$; Table 3.1). This difference was driven by an increase in standing fully in the stall by metritic animals (d -3: $F_{1,28} = 4.31, P < 0.05$; d -2: $F_{1,28} = 15.9, P < 0.001$; d -1: $F_{1,28} = 21.77, P < 0.001$; Figure 3.1) on all 3 d before diagnosis. We observed no differences between the health groups in time spent perching and the total time spent at the stall.

There was no difference in the number of actor replacements between healthy and metritic cows, but there was a tendency on d -3 for healthy cows to be replaced less often ($F_{1,28} = 3.59, P = 0.07$). Cows were displaced out of the two standing positions (perching and standing fully in the stall) at similar frequencies.

Cows with metritis tended to have a higher number of aborted lying events compared with their healthy counterparts on d -2 ($F_{1,28} = 2.99, P = 0.09$), and had more aborted lying events on d -1 ($F_{1,28} = 5.9, P < 0.05$). Cows with metritis also tended to have longer average latencies to lie down than healthy cows on d -2 ($F_{1,28} = 2.84, P = 0.1$). Healthy and metritic cows did not differ in the number of visits to the lying stall.
Table 3.1: Behaviours at the lying stall in the 3 d before diagnosis.

<table>
<thead>
<tr>
<th>Day to diagnosis</th>
<th>Behaviour</th>
<th>d -3</th>
<th>d -2</th>
<th>d -1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>M</td>
<td>SE</td>
<td>H</td>
</tr>
<tr>
<td>Position at stall (min/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perching</td>
<td>230</td>
<td>221</td>
<td>20.6</td>
<td>257</td>
</tr>
<tr>
<td>Lying$^1$</td>
<td>628</td>
<td>613</td>
<td>NA</td>
<td>596</td>
</tr>
<tr>
<td></td>
<td>± 85</td>
<td>± 142.9</td>
<td></td>
<td>± 135.7</td>
</tr>
<tr>
<td>Standing in stall$^2$</td>
<td>261</td>
<td>310</td>
<td>20.5</td>
<td>284$^*$</td>
</tr>
<tr>
<td>Total time in stall$^3$</td>
<td>899</td>
<td>913</td>
<td>31.7</td>
<td>891</td>
</tr>
<tr>
<td>Social interactions (no./d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actor replacements</td>
<td>2.1</td>
<td>2.4</td>
<td>0.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Reactor replacements</td>
<td>1.9$^*$</td>
<td>3.3$^*$</td>
<td>0.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Day to diagnosis</td>
<td>d -3</td>
<td></td>
<td></td>
<td>d -2</td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>M</td>
<td>SE</td>
<td>H</td>
</tr>
<tr>
<td>Reactor position when replaced (no./60 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing fully in stall</td>
<td>1.1</td>
<td>1.4</td>
<td>NA</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>(0.9-1.4)</td>
<td>(1.1-1.8)</td>
<td></td>
<td>(1-1.7)</td>
</tr>
<tr>
<td>Perching</td>
<td>0.5</td>
<td>0.6</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Lying down behaviours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visits to the stall (no./d)</td>
<td>7.6</td>
<td>6.5</td>
<td>1.5</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>(2.2-5.3)</td>
<td>(3.2-7.6)</td>
<td></td>
<td>(2-4.9)</td>
</tr>
<tr>
<td>aborted lying events (no./d)</td>
<td>3.4</td>
<td>4.9</td>
<td>NA</td>
<td>3.2*</td>
</tr>
<tr>
<td></td>
<td>(2.2-5.3)</td>
<td>(3.2-7.6)</td>
<td></td>
<td>(2-4.9)</td>
</tr>
<tr>
<td>Average latency to lie down (min/lying bout)</td>
<td>3.0</td>
<td>5.3</td>
<td>NA</td>
<td>2.7*</td>
</tr>
<tr>
<td></td>
<td>(1.7-5.3)</td>
<td>(3-9.1)</td>
<td></td>
<td>(1.4-5.3)</td>
</tr>
<tr>
<td>Lying bouts (no./d)</td>
<td>15 ± 5.2</td>
<td>14.7 ±</td>
<td>NA</td>
<td>15.1 ±</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td></td>
<td></td>
<td>4.8</td>
</tr>
</tbody>
</table>
Table 3.1 Stall positions, social behaviours and lying down behaviours of healthy (H, $n = 16$) and metritic (M, $n = 16$) primiparous cows in the 3 d before diagnosis. Results are reported as LS means ± SE for normally distributed variables. Non-normally distributed variables ($^4$) were log$_{10}$ transformed for the analysis and reported as back-transformed geometric means and 95 % CI.

1 differences in these outcome measures were previously reported by Neave et al. (2017b); these measures are only reported here (mean ± SD) to supplement outcome measures of interest and were not analyzed statistically.

2 sum of time a cow spent perching and standing fully in the stall

3 sum of time a cow spent perching, standing fully and lying in the stall

4 Not-normally distributed variables; data are reported as back-transformed geometric means and 95 % CI.

$^{a,b}$ different superscripts within a row and day indicate $P \leq 0.05$ between health categories

* raised stars within a row and day indicate $P \leq 0.1$ between health categories

---

**Figure 3.1 Daily time metritic and healthy cows stood fully in the stall.**

Standing fully in the stall in metritic (M, $n = 16$) vs. healthy (H, $n = 16$) primiparous dairy cows in the 3 d before diagnosis. Whiskers show 1.5 × interquartile range, boxes show interquartile range, the white line shows the median, the open rectangles show the mean, and the filled triangles show outliers (in the healthy cow group, the outlier was to the same cow on all 3 d and in the metritic cow group the outliers are from 2 different cows).
3.4 Discussion

As predicted, primiparous cows diagnosed with metritis spent more time standing in the stall. This difference became more pronounced over the 3 d observation period, possible due to the progression of metritis. The difference in standing time in the stall was driven by prolonged standing with all 4 feet in the stall by the metritic cows. Previous work has shown that standing in the stall with all four feet is dependent on stall design (Tucker and Weary, 2004; Tucker et al., 2005); for example, when the neck rail is placed in a less restrictive position at 190 cm distance to the rear curb, cows increase the time they spend standing fully in the stall (Fregonesi et al., 2009). Interestingly, these authors reported that the early-lactation healthy cows used in their study only spent approximately 36 min/d engaged in this behaviour. In contrast, the sick primiparous cows in our study stood more than twice as long with 4 feet in the stall, despite the presence of a more restrictively placed neck rail (165 cm from the rear curb, horizontally measured). This difference is consistent with the idea that cows with metritis are more highly motivated to perform this behaviour.

One response to sickness in social animals is self-isolation (Hart 1988). Proudfoot et al. (2014) reported that, in contrast to healthy cows, freshly calved dairy cows diagnosed with mastitis or metritis spent more time behind a plywood board that provided a visual barrier from the herd. Similarly, sick calves were found to lie down further away from pen mates than healthy ones (Cramer and Stanton, 2015; Cramer et al., 2016). Cows in our study were not able to visually or physically separate themselves from the group, but spending time standing in the stall may have provided some form of protection. DeVries and von Keyserlingk (2006) and Jensen et al. (2008) provide evidence that partitions at the feeder reduce competitive behaviours in cows and calves, respectively. We suggest that the increased time spent standing fully in the stall can
be viewed as a type of self-isolation sickness behaviour. However, given the influence of stall design on this behaviour, future work is needed to investigate how stall dimensions and cow size influence this change in behaviour. In addition, we may have gained a better understanding of when stall standing behaviours change in relation to clinical symptoms with a daily examination of vaginal discharge as opposed to the 3 d interval.

Ill animals typically increase resting time, likely to conserve energy and support the systemic response to infection (Hart, 1988). However, as reported in our companion paper (Neave et al., 2018), we noted no differences in lying time between metritic and healthy cows. We hypothesized that this might be a consequence of the sick cows being hesitant to lie down, possibly in response to visceral pain (Stojkov et al., 2015), as longer standing times have also been reported after liver biopsy (Mølgaard et al., 2012) and ruminal acidosis (DeVries et al., 2009). We therefore predicted that metritic cows would show more behaviours indicating a reluctance to lie down, as measured by aborted lying events. Similar abnormal lying down movements as described in our study have been used by others to measure stall comfort (Krohn and Munksgaard, 1993; Tucker and Weary, 2004; Dippel et al., 2009), as well as responses to different milking regimes (Österman and Redbo, 2001) and oligo-fructose overload (Niss et al., 2009). We suggest that pain due to lying down may explain the health group differences in the current study, especially since these differences became more pronounced with time. Visceral inflammatory pain can be referred to other viscera or somatic areas (viscero-visceral or viscero-somatic pain; Gebhart and Bielefeldt, 2016). Visceral pain and viscero-visceral pain likely contributed to increased back arch during transrectal uterine palpation in metritic versus healthy cows (Stojkov et al., 2015). In the current study, referred pain to the viscera may have resulted in pain during lying down movements that put tension or pressure on the viscera. Visceral pain may
have also led to somatic hyperalgesia (e.g. Wesselmann and Lai, 1997), making skin areas sensitive to touch during lying down movements and when lying. We encourage future studies to include measures of hyperalgesia in dairy cows with uterine infection.

The observed reluctance to lie down, measured by aborted lying events, may explain why primiparous cows with metritis had shorter lying times than expected given their illness (Neave et al., 2018). This may also have contributed to the increase in standing fully in the stall by animals with metritis; despite no differences in latencies to lie down, metritic cows may have initially searched for a suitable lying space but then remained standing in the stall.

Avoidance of competitive interactions at the feed bunk has been reported in dairy cows before diagnosis with metritis and mastitis (Huzzey et al., 2007, Sepúlveda-Varas et al., 2016), providing the basis for our prediction that metritic cows would take part in fewer agonistic interactions at the lying stalls. However, we found little evidence of a difference between health groups. The high number of actor replacements in ill cows in our study may be explained by increased motivation of these animals to access the lying stalls, resulting in increased time spent fully standing in the stall.

The combined number of actor and reactor displacements from the lying stalls in the current study was higher than that reported by others using similar stocking densities (Fregonesi et al., 2007; Val-Laillet et al., 2008; Winckler et al., 2015). These previous studies used mid-lactation, healthy cows housed in stable groups. The higher replacement rate in our study may be explained by the frequent regrouping due to the dynamic group structure. In addition, we only observed primiparous cows that were likely at greater risk of being replaced than multiparous animals as these animals likely had a lower rank within the group hierarchy. Wierenga (1990) reported that younger cows were replaced more often at the feeder and at the lying stalls.
Interestingly, standing in the stall in either position (perching or standing fully in the stall) did not seem to provide protection for the cows with metritis, given that they were displaced similarly often as healthy cows. A growing body of literature describes behavioural changes before diagnosis of clinical disease in dairy cows, but much of this focuses on DMI and feeding behaviours. With the development of technology that allows for real-time tracking of cow location within the pen (Meunier et al., 2017; Wolfger et al., 2017) and real-time detection of lying behaviour (Porto et al., 2013), it is now also feasible to use behavioural changes at the lying stalls.

3.5 Conclusions

Metritic cows spent more time standing in the stall. Behavioral differences may have been part of a general response to sickness or may be specifically associated with pain, as suggested by an increased number of aborted lying events. Our findings suggest that increased times standing in the stall may be helpful in identifying sick dairy cows.
Chapter 4: Behavioural changes associated with fever in transition dairy cows

A version of this chapter has been submitted for publication: J. Lomb, M. A. G. von Keyserlingk and D. M. Weary. Changes in behaviour in dairy cows with elevated body temperature.

4.1 Introduction

There is a high incidence of illness in the weeks around calving (Mulligan and Doherty, 2008; LeBlanc, 2010). Early detection of illness may be accomplished by different strategies, including changes in behaviour; behavioural changes associated with infectious disease have been described across a variety of species and these are typically referred to as ‘sickness behaviours’ (Hart, 1988; Shakhar and Shakhar, 2015). These changes include reduced feed intake, reduced social interactions and reduced activity, as described in transition cows in the days leading up to clinical diagnosis of metritis and mastitis (Sepúlveda-Varas et al., 2016; King et al., 2018; Neave et al., 2018). Assessing sickness related behavioural changes, either by comparing behaviours within individuals over time, or by comparing animals with a healthy control group, has been suggested as a strategy to identify ill cows in early lactation (King et al., 2018).

During transition, many cows show evidence of fever (i.e. rectal body temperature (BT) > 39.5 °C, Suthar et al., 2012) without other signs of illness (Wenz et al., 2011; Sutherland et al., 2012). It is difficult to classify such individuals as either sick or healthy. Fever and other behavioural signs of sickness are often closely related and mediated through similar immunological and hormonal pathways (Bleeker-Rovers et al., 2009; Pecchi et al., 2009). For example, research has shown that mid-lactation cows challenged with intravenous
lipopolysaccharides (LPS) exhibit both reduced DMI and fever (Waldron et al., 2003; Zhao et al., 2018). However, little work has disentangled the relationship between fever and sickness behaviours in transition dairy cows, specifically focusing on animals showing signs of fever but no other clinical signs of illness.

The objectives of this study were to describe changes in feeding, social and lying behaviours of transition dairy cows with fever (defined as rectal BT > 39.5 °C) but not showing other signs of clinical disease. We hypothesized that, on days that fever was recorded, cows would express sickness behaviours and that these changes would be greater when a cow had multiple days of fever.

4.2 Methods

The data presented in this study was also generated by the study presented in Chapter 2 (published: Lomb et al., 2018a). Again, the methods are briefly described here and additions made where appropriate.

4.2.1 Animals, Management and Housing

During the study period the health and behaviours of all initially healthy and non-lame primiparous (n = 105) and multiparous (n = 232) Holstein cows were monitored from approximately 3 wk before to 3 wk after calving, when cows were housed in an experimental prepartum and postpartum pen depending their gestational and lactational status. Both pens were fitted with 12 electronic feed bins (Insentec, Marknesse, Holland), 2 electronic water bins of the same system, and 24 lying stalls. The lying stalls were equipped with mattresses (Pasture Mat, Promat Inc., Woodstock, Ontario, Canada) covered with approximately 5 cm of sand. At all times, stocking density in both pens was maintained at 20 cows. When a pregnant cow in the
prepartum pen showed imminent signs of calving (i.e. relaxation of tail ligament, milk let down), she was moved to a designated calving pen. Within 24 h after calving, she was then moved to the postpartum pen. The pen movements of the calving cow coincided with one pregnant cow being moved to the prepartum pen, and the cow with the highest DIM being moved out of the postpartum pen. The calving pen contained a sawdust open pack and cows had access to 1 electronic water and 6 electronic feed bins, of which 1 was filled per cow in the pen (max. 2 cows at the same time).

Cows in both pens were fed ad libitum a TMR meeting or exceeding NRC (2001) requirements. The pre-partum cow TMR included 32% corn silage, 37% alfalfa hay, 18% rye grass straw and 13% concentrate (DM: 52.4 ± 4.7%, CP: 14.3 ± 0.34% DM, ADF: 34.6 ± 0.60% DM, NDF: 46.5 ± 0.17% DM, and NE\textsubscript{L}: 1.39 ± 0.0071 Mcal/kg). The post-partum cow TMR included 26% corn silage, 13% grass silage, 7% alfalfa hay, 4% grass hay and 50% grain concentrate mash (DM: 50.35 ± 2.5%, CP: 18.3 ± 0.58% DM, ADF: 18.1 ± 0.71% DM, NDF: 28.5 ± 1.2% DM, and NE\textsubscript{L}: 1.72 ± 0.014 Mcal/kg). Fresh feed was delivered twice daily, at approximately 0800 and 1600 h. After calving, cows were milked twice per day at 0700 and 1700 h.

After calving, cows were screened for health and all signs of illness were recorded. For the purpose of metritis examination, cows were retained in a sorting area after morning milking (between 0800 and 1000 h) on every third day after calving, beginning at 3 DIM until 21 DIM. Metritis was diagnosed by the researchers, overseen by a trained veterinarian, based on vaginal discharge, collected with a gloved hand and scored on a scale from 0 to 4, where 0 = healthy, and 4 = severe metritis (i.e. watery, foul-smelling discharge; Urton et al., 2005; Huzzey et al., 2007).
Farm staff monitored all cows for other transition cow diseases, including ketosis, downer cow syndrome and displaced abomasum, and recorded and treated these according to farm protocol. Details on farm protocols to identify ill cows are described by Neave et al. (2017). Briefly, cows were screened for mastitis twice daily during regular milking routine, based on appearance of the udder and milk quality. When a cow presented with reduced feed intake and milk yield, milk strips (KetoTest, Elanco Animal Health, Nagoya, Japan) were used to confirm ketosis. Displaced abomasum was diagnosed by the herd veterinarian after farm staff had identified cows with a typical metallic sound when auscultating the left flank.

During the first 21 DIM, rectal temperature was either taken when cows were restrained in head locks during health checks (every 3 DIM between 0800 and 1000 h), or in the pen, when most cows were settled in the lying stalls after morning milking (all other days between 1000 and 1200 h). When taken during the health check, BT was taken before any other procedures were performed. BT was measured using an electronic thermometer (Nexcare™ Rapid Digital Thermometer, 3M, St. Paul, MN) inserted ~ 10 cm and ensuring contact with the rectal wall. The measurement was taken when an acoustic signal was given by the thermometer. BT was taken by multiple observers (n > 5) who all were trained and monitored by one of the 2 main researchers when performing the procedure during the health check and in the pen. BT measures (measured as rectal temperature) by different trained observers are known to be highly correlated (r = 0.96; Burfeind et al., 2010).
4.2.2 Study animals

**Cows with fever**

All cows that were clinically healthy (i.e. had no signs of disease at any point during the first 21 DIM) but had at least one day of fever (BT > 39.5°C) were eligible to be part of the study. Expecting that cows with multiple days of fever would show a greater change in behaviours, we distinguished between cows that had 2 or more consecutive days of fever (n = 8), and those with one day of fever (n = 18). Within the 21 d period, most cows had missing BT data. Out of the 8 cows with multiple days of fever, 4 cows had 1 d of missing BT data (BT missing before d of fever: n = 2; BT missing after d of fever: n = 2), and 3 cows had 2 d of missing BT data (after fever: n = 2; before and after fever: n = 1). Out of the 18 cows with 1 d of fever, BT data were not available for 11 cows; 1 d of BT records was missing for 6 cows (before fever: n = 1; after fever: n = 5), and 2 d of BT records were missing for 5 cows (before fever: n = 2; after fever: n = 1; before and after fever: n = 2).

**Control cows**

Given that feeding and lying behaviours are highly variable in the first weeks of lactation (Huzzey et al., 2005; Kaufman et al., 2016; Neave et al., 2017), we compared each fever cow with one healthy cow based on DIM. For each cow with fever the next cow that calved was selected, provided that (1) she was healthy (i.e. not diagnosed with a clinical disease) and she did not have a fever (BT ≤ 39.5°C), (2) she was in the same parity group (i.e. primiparous or multiparous), and (3) that there were no more than 2 d of missing BT. Of the 8 controls for cows with multiple days of fever, 1 d of BT data was missing for 3 cows. Of the 18 control cows for cows with 1 d of fever, 1 d of BT data was missing for 4 cows and 2 d of BT data were missing.
for 1 cow. Throughout the text, controls are referred to as ‘healthy cows’, and details on both health groups are summarized in Table 4.1.

**Table 4.1 Details on cows with fever and healthy cows.**

<table>
<thead>
<tr>
<th></th>
<th>Multiple days of fever</th>
<th>One day of fever</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fever</td>
<td>Healthy</td>
</tr>
<tr>
<td>Multiparous (no.)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Primiparous (no.)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BT (°C)</td>
<td>39.9 ± 0.3</td>
<td>38.7 ± 0.5</td>
</tr>
<tr>
<td>DIM (d)</td>
<td>6.4 ± 3.5</td>
<td>6.4 ± 3.5</td>
</tr>
<tr>
<td>Ambient temp. (°C)</td>
<td>25.9 ± 5.2</td>
<td>21.2 ± 6.7</td>
</tr>
</tbody>
</table>

The number of multiparous and primiparous cows with one day and multiple days of fever, and healthy control cows matched by DIM. The mean (± SD) body temperature (BT), days in milk (DIM), and ambient temperature (temp.) during the days considered, are shown separately for each health category.

### 4.2.3 Behavioural measures

Feeding behaviours were measured using the electronic feed bins. Cows were fitted with ear transponders that registered when a cow entered a specific bin and recorded the difference in weight of feed from when she entered and exited the bin (described and validated by Chapinal et al., 2007). From this data we calculated DMI (kg/d), feeding time (min/d) and the number of times a cow entered a feed bin (i.e. visits) per day. We further calculated the number of daily meals with a new meal being recorded when a cow had not visited the feeder for a minimum of 20.1 min (Proudfoot et al., 2009b). Lastly, we calculated the feeding rate as g DMI per min spent at the feed bunk.
The data from the electronic feed bins were also used to estimate the number of competitive displacements from the feeder. A displacement was defined as one cow (i.e. actor) forcefully pushing another cow (i.e. reactor) out of a feed bin. As validated by Huzzey et al. (2014), a threshold of 26 sec between 2 consecutive visits at one feed bin was used to identify a displacement.

Lying behaviours were monitored using electronic data loggers (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA) fitted onto a hind leg using elastic bandages (Co-Flex, Andover Coated Products Inc., Salisbury, MA). Loggers were set to record the g-forces on 2 axes (y- and x-axis) at 1-min intervals. Data were downloaded weekly when loggers were changed and summarized into daily lying behaviour (i.e. lying time (min/d), number of lying bouts (no./d) and average lying bout duration (min/bout)) using an algorithm in SAS developed by UBC’s Animal Welfare Program (2013) using the cut-off described by Ledgerwood et al. (2010).

4.2.4 Statistical analyses

All statistical analyses were performed using SAS (Version 9.4; SAS Institute Inc., Cary, NC). Behaviours were summarized by cow and day. All variables were scrutinized using PROC UNIVARIATE.

A general linear mixed model (PROC MIXED) was used for the analysis of behavioural differences between health groups, with cow as the experimental unit. Cows with multiple days of fever and cows with one day of fever, DIM of fever (as continuous variable), health status (fever vs. healthy) and the interaction of DIM of fever with health status were offered to the model as fixed effects. Parity (primiparous vs. multiparous) was only included as fixed effect for
the analysis of cows with 1 d of fever as there were no primiparous cows with multiple days of
temperature, obtained through records provided by the
Environment Canada weather station in Agassiz, was offered to both models, but did not account
for variation in outcome measures in either model and was therefore omitted.

For the analysis that addressed behavioural differences in cows with multiple days of
fever, the first 2 days of fever were analyzed. As the interaction of health status and day of fever
was not significant, behaviour values were averaged to one data point for each cow.

The significance level was set at $P \leq 0.5$, and a tendency at $P \leq 0.10$. Interactions were
retained in the model when $P < 0.1$.

4.3 Results

4.3.1 Cows with multiple days of fever

Cows with multiple days of fever had lower DMI ($F_{1,12} = 6.75, P = 0.02$; Figure 4.1), but
there was an interaction between this effect and DIM of fever ($F_{1,12} = 5.86, P = 0.03$), such that
at lower DIM cows with fever ate less and at higher DIM cows with fever ate more than healthy
cows. Cows with fever also spent less time at the feed bunk ($F_{1,13} = 22.88, P < 0.001$; Figure 4.1)
than cows without fever. Cows with fever tended to eat at a faster rate ($F_{1,12} = 3.6, P = 0.08$;
Figure 4.1), and there was an interaction between fever and DIM ($F_{1,12} = 14.75, P < 0.01$) due to
cows with fever eating faster with increasing DIM, and healthy cows eating more slowly with
increasing DIM. There was no effect of fever on feeder visits (Figure 4.2), meals and
displacements from the feed bunk (Table 4.2).
Figure 4.1 Feeding behaviour differences on the day(s) of fever.

Least squares mean (± SE) DMI (kg/d), feeding time (min/d) and feeding rate (g/min) for cows with multiple days of fever ($n=16$; averaged for the first 2 d of fever) and one day of fever ($n=32$) compared with healthy cows.
Cows with fever had shorter lying times than healthy cows \((F_{1,13} = 13.54, P < 0.01;\) Figure 4.2). Cows with fever and healthy cows had a similar number of lying bouts, and there was no effect of fever on lying bout duration.

**Figure 4.2 Feeder visits and lying time on the day(s) of fever**

Least squares mean (± SE) visits to the feeder (no./d) and lying time (h/d) for cows with multiple days of fever \((n = 16;\) averaged for the first 2 d of fever) and one day of fever \((n = 32;\) lying time: \(n = 28\) ) compared with healthy cows.
Table 4.2 Behaviour differences on the day(s) of fever.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Multiple days of fever</th>
<th>One day of fever</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fever</td>
<td>Healthy</td>
</tr>
<tr>
<td>Meals (no./d)</td>
<td>11.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Actor replacements (no./d)</td>
<td>8.3</td>
<td>10.1</td>
</tr>
<tr>
<td>Reactor replacements (no./d)</td>
<td>8.3</td>
<td>11.4</td>
</tr>
<tr>
<td>Lying bouts (no./d)</td>
<td>9.7</td>
<td>12.0</td>
</tr>
<tr>
<td>Lying bout duration (min/bout)</td>
<td>63</td>
<td>66.1</td>
</tr>
</tbody>
</table>

Least squares mean (± SE) number of meals (no./d), social interactions (showing actor and reactor separately; no./d), lying bouts (no./d) and lying bout duration (min/bout) for cows with multiple days of fever ($n = 16$; average of the first 2 d of fever) and one day of fever ($n = 32$; lying behaviours: $n = 28$) compared with healthy cows.

4.3.2 Cows with one day of fever

Cows with one day of fever spent less time at the feed bunk ($F_{1,32} = 4.0$, $P = 0.05$; Figure 4.1) and had fewer visits to the feed bunk ($F_{1,31} = 5.78$, $P = 0.02$; Figure 4.2) than healthy cows.

For feeder visits, there was a tendency of an interaction between fever and DIM ($F_{1,31} = 3.02$, $P = 0.09$), such that the effect of fever was greater at early DIM. Cows with fever had a faster feeding rate than healthy cows ($F_{1,32} = 6.88$, $P = 0.01$; Figure 4.1). There was no effect of fever on DMI or number of meals.
Cows with fever were involved in fewer social interactions; they were less likely to displace other cows from the feed bunk \((F_{1,32} = 4.29, P = 0.05)\) and were less likely to be replaced by other cows \((F_{1,32} = 4.39, P = 0.04)\) relative to healthy cows (Table 4.2). There was no effect of fever on lying behaviours (Figure 4.2).

### 4.4 Discussion

Cows with fever (either on a single day or more) showed behaviours consistent with sickness behaviours expressed during clinical disease. However, these behavioural differences were not consistently more pronounced in cows with multiple days of fever than in cows with one day of fever. We had expected that cows showing multiple days of fever would show the greatest response. Other work has shown that disease severity is associated with sickness responses. For example, rats infected with brewer’s yeast showed a dose-dependent fever and behavioural response, in that fever and behavioural changes (i.e. anorexia, lethargy) lasted longer and were greater in those animals receiving higher doses of the infecting agent (Dangarembizi et al., 2018). Some of the inconsistencies noted in our study between one day versus multiple days of fever may have been due to differences in group composition and environmental temperature. All cows with multiple days of fever were multiparous; whereas, cows with one day fever included primiparous cows, likely affecting the results (see Neave et al., 2017). The average ambient temperatures were also higher on the days included in the analysis of multiple days of fever, and heat stress is known to affect feeding (Allen et al., 2015) and standing behaviour (Cook et al., 2007).

Our most pronounced and consistent finding was that cows with fever (on one or more days) spent less time feeding. Even though feeding time and DMI are related (Johnston and
Devries, 2018), the effect on this behaviour was only significant for cows with multiple days of fever. Anorexia is considered a classical sickness behaviour in many species (Hart, 1988; Tizard, 2008), and has been shown in dairy cows after LPS challenge (Waldron et al., 2003; Zhao et al., 2018). Our findings suggest that a short-lasting fever may not lead to anorexia in dairy cows, and that reduced feeding time may be a more sensitive indicator of fever than reduced DMI. Decreased feeding is also consistent with a more general decrease in activity, as commonly seen in sick animals (Hart, 1988). In the current study we did not include other measures of activity, such as daily step count, but encourage these to be considered in future work.

The combination of decreased feeding time, higher feeding rate, and reduced competitive interactions at the feed bunk in cows with one day of fever, may suggest that fever cows experienced increased social stress at the feeding area. In competitive environments cows spend less time feeding (DeVries et al., 2004) and compensate by eating at a faster rate (Nielsen, 1999; Proudfoot et al., 2009b). Reduced competitive interactions were also observed in cows with clinical mastitis, explained in part by these cows avoiding the feed bunk during peak feeding times when competition was greatest (Sepúlveda-Varas et al., 2016).

Even though cows with fever were less active at the feed bunk, they did not spend more time lying down. Indeed, cows with multiple days of fever spent less time lying down (and had fewer lying bouts) compared with healthy animals. The elevated ambient temperatures that some of the cows experienced may have impacted these results (e.g. increased standing times associated with heat stress; Cook et al., 2007). We suggest that future research investigate sickness behaviours during periods of heat stress, as heat stress may result in similar behaviours, reducing the usefulness of these behaviours for identifying ill animals in hot weather.
Not all behavioural changes observed in our study were in line with changes previously described in transition cows with clinical infectious disease. For example, previous work has shown that cows with metritis and mastitis (with or without fever) consume less feed and engage in fewer social interactions in the days before diagnosis (Sepúlveda-Varas et al., 2016; Neave et al., 2018). However, cows with mastitis decreased feeding rate in the days before diagnosis (Sepúlveda-Varas et al., 2016) as opposed to the increased feeding rate of cows in our study, and cows with metritis had similar feeding times as healthy cows (Neave et al., 2018), not shorter feeding times as evidenced in the current study. Some such maladies may be painful (Fitzpatrick et al., 2013; Stojkov et al., 2015) and this pain may result in behavioural change (Weary et al., 2006), possibly altering the expression of sickness behaviours. Future studies should attempt to isolate the effect of pain, for example by comparing sick animals with and without treatment using effective analgesics.

The availability of sensors that automatically record feeding behaviour, including feeding time and time spent in proximity to the feed bunk (Borchers et al., 2016; Benaissa et al., 2017) have greatly expanded the types of data that can now be collected on farms; the results of the current study suggest that changes in such automatically recorded measures would detect transition cows with fever. Given that the fever resolves within a short period, these cows may not need treatment; instead the findings of the current study may contribute to the knowledge needed for improving accuracy in detection transition cows with clinical disease.
4.5 Conclusions

Cows with fever differed in feeding and social behaviors relative to healthy controls; some of these differences are consistent with the expression of sickness behaviors expressed during responses to clinical disease.
Chapter 5: General discussion and conclusions

5.1 Findings, implications and future research

In Chapter 1, I reviewed literature on transition cow health and its association with behaviour. Intrinsic and extrinsic risk factors lead to a high number of cows becoming sick in the weeks around calving, with metritis being one of the most commonly diagnosed disease. Ill cows experience reduced welfare through the impairment of biological functioning, but also through the association of disease with malaise and pain. Sickness induced behavioural changes, typically referred to as sickness behaviours, have been described in many species and to a limited extent in dairy cows. In this dissertation I argued that sickness behaviours can be used as a tool for early identification of sick cows, as has been suggested by others, but also that understanding sickness behaviours will lead to an improved understanding of sick cows’ environmental needs. Collectively this information will enhance our ability to evaluate the effects that medical treatment has on the affective state of the cow.

This last idea was addressed in Chapter 2 and served as basis for my thesis from where the consequent studies evolved. In Chapter 2, we investigated the effects of meloxicam on a suite of behaviours in cows diagnosed metritis. NSAID like meloxicam reduce the expression of the central hormone regulating the sickness response (PGE$_2$; Pecchi et al., 2009). A reduction of sickness behaviours after NSAID administration had been shown in laboratory rodents challenged with LPS (Harden et al., 2015). To investigate the effects of meloxicam we conducted a blinded randomized controlled trial, a high-ranked methodology for evaluating the effectiveness of medical treatment (Evans, 2002). A second strength of the study was that the design came close to current methods of metritis treatment on farms; a recent study exploring metritis treatment on Californian dairy farms reported that 42 % of farms used an NSAID
(typically acetyl salicylate flunixin meglumine) as adjunctive treatment to antimicrobials (Espadamala et al., 2018).

Unfortunately, we observed an inconsistency of behavioural change after treatment with some behaviours improving more in cows that the received the placebo, and other behaviours improving more in cows receiving meloxicam. This created some difficulty for drawing conclusions about a reduction of sickness behaviours due to meloxicam treatment in our study. Possible reasons for the inconclusive results are elucidated in the discussion of Chapter 2, for example the additional administration of antimicrobials. Given that different NSAID vary in some of their pharmacological characteristics (e.g. tissue distribution and central vs. peripheral mechanisms; Engelhardt, 1995; Burian and Geislinger, 2005), caution is warranted when extrapolating the results of our study to the use of other NSAID.

One of the limitations of the Chapter 2 study was the lack of pain-specific measures that would have allowed more direct assessment of the analgesic efficacy of meloxicam. Understanding the analgesic effects on pain related behaviours may have helped in interpreting which of the observed behavioural changes were due to reduced pain and which were due to reduced malaise. For example, we hypothesized that analgesic effects were more profound in primiparous than in multiparous cows, as there were parity differences in some outcome measures after meloxicam treatment (e.g. increased vs. decreased lying time in multiparous vs. primiparous cows). Behaviours directly linked to a painful stimulus or condition may have allowed for verifying this hypothesis. Unfortunately, at the outset of the study no pain-specific behaviours for cows with metritis had been described. In 2015, Stojkov et al. provided the first evidence of visceral pain in cows with metritis, and, equally important, a way to assess this (via the back arch provoked through rectal pressure on the uterus). In the same year, Gleerup et al.
(2015) described a ‘pain face’ in cows with lameness, which may also be observable in cows with metritis. I believe that especially including a measure of arched back would have provided substantial information on the analgesic effect of meloxicam and aided with the interpretation of our results.

Despite the inconclusive results reported in Chapter 2, this study demonstrates how behaviour change may be used to assess affective states of metritic cows after treatment. I argue that this information will be important as we continue to work on reducing the suffering associated with ill health in cows. The high self-recovery rate of metritis (McLaughlin et al., 2012), combined with growing concerns about anti-microbial use in production animals (Van Boeckel et al., 2015), has led some to argue that treatment should be delayed in hope that the sick animal will spontaneously recover (Sannmann et al., 2013) or that alternative treatment methods could be used (Haimerl and Heuwieser, 2014). While these may result in reduced use of antimicrobials, the welfare consequences for affected cows will have to be considered and behavioural evaluations may be best suited for doing so.

The inconsistencies of behavioural change that were observed after metritis treatment between the treatment groups resulted in our inability to show a clear of benefit of the NSAID treatment. This prompted us to investigate sickness behaviours before diagnosis and treatment in more detail. Given the finding of Chapter 2 that primiparous had increased lying time after meloxicam treatment (as opposed to a decrease in lying time, as expected, given that sickness is typically associated with increased resting), the study presented in Chapter 3 focused on lying associated behaviours and behaviours at the lying stall in the days before primiparous cows were diagnosed with metritis. The main findings highlighted in this chapter were that metritic
primiparous cows spent more time standing fully in the stall than healthy animals, and showed a higher number of aborted lying events, likely indicative of a reluctance to lie down.

Aborted lying events have been used by others to assess lying comfort (e.g. Dippel et al., 2009) and pain (Niss et al., 2009). Our definition of aborted lying events varied slightly from what has been previously described given that we assessed behaviours from video recordings that were not made with this measure in mind. However, by adding additional behavioural criteria (i.e. leg movements towards the center of the cow’s body) to earlier definitions (e.g. sweeping head movements: Österman and Redbo, 2001; Tucker and Weary, 2004) we were able to reach high inter-observer and intra-observer agreement (Kaufman and Rosenthal, 2009), one of the key requirements when using subjective behavioural measures (Weary et al., 2006). In the context of what we observed in Chapter 2 (i.e. increased lying time after meloxicam treatment), and with other reports in mind (Stojkov et al., 2015) that suggested that cows with metritis experience pain, we interpreted the higher number of aborted lying events in primiparous metritic cows as associated with pain from the metritis infection. To confirm that this was indeed the case, we would have ideally studied the effect of analgesic treatment on the occurrence of the behaviour in metritic cows and compared these results to a healthy control cohort that also received the analgesic treatment (Weary et al., 2006). Unfortunately, we were not able to study the effects of meloxicam and placebo treatment within the metritic cow group due to insufficient statistical power; too few heifers were diagnosed with severe metritis (which was our inclusion criteria for this study) and this was further complicated by the fact that there was a baseline difference observed between placebo and meloxicam treated heifers for some of the investigated parameters. However, these findings support that future research should address analgesic treatment in cows with metritis.
The observation that primiparous cows with metritis spent more time standing fully in the stall drew our attention to the social aspect of the sickness response. Some authors argue that social distance to conspecifics may be the most important function of the sickness response given that this reduces pathogen distribution; nearly all sickness behaviours can be interpreted as a form of reduction of contact to the social group (Shakhar and Shakhar, 2015). The observational nature of the study does not allow us to draw strong conclusions about the actual motivation for metritic cows to stand longer fully in the stall, and thus our interpretation that the heifers sought to create distance to the group remains speculative. However, at the very least the findings in Chapter 3 demonstrate how very little we know about the environmental needs of ill cows.

Common current practice is to place sick cows in a designated pen, but farmers rarely consider metritis a malady warranting this special treatment (Fogsgaard et al., 2016). On many farms ill cows are co-mingled with other animals (e.g. fresh cows, dry cows; Fogsgaard et al., 2016) in the sick pen. There is little evidence of beneficial or detrimental effects of moving ill cows to a sick pen, but introducing ill cows to a new group may be an additional stressor given its negative effects on behaviour (von Keyserlingk et al., 2008; Schirmann et al., 2011). I suggest that future research investigate the role of environmental management of sick cows generally and the motivation of sick cows to self-isolate specifically.

The findings arising from the study presented in Chapter 3 also support the idea that sickness related behavioural changes are observable before clinical symptoms appear. The fact that metritis was assessed on every third day after calving as opposed to every day, and only the 3 days before diagnosis were included in the study, prevented an exact analysis of when behaviours, e.g. standing fully in the stall, first differ between healthy and sick cows in relation to the day when vaginal discharge was first scored positive for metritis. However, when our
results are taken together with other evidence (e.g. Sepulveda-Varas et al., 2016; King et al., 2018), the evidence suggests that the early identification of sick transition cows will be possible by following changes in sickness behaviours. Consequently, one then must ask which actions should be taken to best care for these cows. Sickness behaviours are not disease specific but rather part of the general innate immune response (Pecchi et al., 2009). For example, if the behaviour of standing fully in the stall was motivated by a need for seeking self-isolation by the metritic cows, it is likely that this behaviour is not specifically associated with metritis but rather with malaise which can arise from a number of different diseases. Thus, future research needs to address which actions need to be taken when ill cows are identified but where a clinical diagnosis is not yet possible. To complicate this further, Chapter 4 presents some evidence that not all cows that display sickness behaviours, i.e. cows with one or more days of fever but without clinical disease, will necessarily need treatment.

The motivation for conducting the study presented in Chapter 4 was to gain a better understanding of sickness behaviours in transition cows, potentially without the influence of localized pain. We thus investigated behavioural change in cows with fever (but without other clinical signs of disease), as fever is closely linked to the behavioural sickness response (Hart 1988; Pecchi et al., 2009). The study revealed that cows with only a fever showed changes in feeding behaviours that were consistent with a sickness response.

The findings presented in this chapter suggest that there is potential for substituting cow-side fever measures with measures of behavioural change. Elevated rectal temperature is frequently used to monitor transition cow health (Smith and Risco 2005; Espadamala 2016), but cow-side fever measures, as well as other health evaluations, require cows to be restrained in headlocks (Espadamala et al., 2016); thus, shortening the time available for other primary
behaviours such as lying and feeding. To further refine the use of behaviour-based technologies to detect cows with fever, future research should address how the expression of sickness behaviours changes with increasing body temperature. Some research in laboratory rats has shown that both, fever and the expression of sickness behaviours increase with increasing dose of infectious agent (Dangarembizi et al., 2017), but others have reported that the expression of fever and sickness behaviours were not always expressed to the same extent (e.g. rat breeds that had a high fever response showed fewer sickness behaviours; Martin et al., 2008).

Some argue that cows with a fever but that are otherwise healthy should not treated with antimicrobials (Suthar et al., 2012). However, cows with fever are likely experiencing a feeling of malaise (explaining the behavioural differences we report) and thus should be worthy of consideration. In Chapter 2, we argued that this sickness-related malaise should be considered a negative affective state and thus a welfare concern requiring intervention. Therefore, the question arises if cows with fever should at least be provided with treatment that reduces malaise, e.g. administered an NSAID, even though fever (Roth and Blatteis, 2014) and sickness behaviours (Dantzer and Kelley, 2007) can support the immune response. Research from humans and laboratory rodents addressing the role of fever and the benefits of NSAID treatment show inconsistent results, with both beneficial and detrimental effects of NSAID treatment observed (Harden et al., 2015).

Fever may have a number of causes of which infection is only one (accounting for only 50 % of fever in humans; Roth and Blatteis, 2014). In clinically healthy transition cows, causes for cytokine-associated inflammatory processes possibly resulting in fever may be gut-derived endotoxins (Eckel and Ametaj, 2016), or non-infectious causes such as tissue damage. There is also a chance that we included false positives in the fever group (i.e. cows with elevated body
temperature not caused by a cytokine-related inflammatory process). Assessing body
temperature only once daily may have led to measurement error; there is some evidence
suggesting that in a small number of cases, the procedure itself of taking rectal temperatures may
cause a difference in body temperature of 0.5°C, even when used with a standardized protocol
(Burfeind et al., 2010). In addition, the high ambient temperatures that some of the study animals
experienced may have increased the risk that we included cows in the fever group that were
hyperthermic (i.e. had a body temperature that exceeded the cow’s thermo-regulatory set-point)
but did not have a fever (i.e. increased body temperature due to an increased thermo-regulatory
set-point). This risk may have been somewhat limited as we consistently measured rectal
temperature before noon when body temperatures are lower (Suthar et al., 2012).

One limitation of my thesis was that all studies were based on the same data set that arose
from a study that was specifically designed for testing the effects of meloxicam in cows with
metritis. Management (e.g. regrouping due to dynamic group), as well as the physical
environment (e.g. freestall, bedding type) and social (e.g. comingling of primiparous and
multiparous cows) environment all influence dairy cow behaviour (e.g. Schirmann et al., 2011;
Campler et al., 2018) and thus likely impacted the expression of sickness behaviours; an
observation also made in other species (Hennessey et al., 2014). Thus, the results of the 3
different studies are not entirely independent from one another, and extrapolation to other dairy
settings should be done with caution. Using the data set from the first study also limited the
methodology for the two subsequent studies. For example, daily metritis checks would have
allowed for a better assessment of the temporal relationship of onset of some behavioural
changes (e.g. standing fully in the stall, Chapter 3) and a different, perhaps continuous fever
assessment would have allowed for more reliability of identification of the cows (Chapter 4).
Lastly, addressing the stated 3 research questions by use of only one data set increased the risk of Type I error (i.e. false positive findings) given the high number of comparisons made. However, we acknowledged this limitation to some extent in Chapter 2, by providing information on the predictions as stated in the original study proposal (see Table 2.4). In addition, using this data set made it possible to explore ideas that arose out of the first study (e.g. standing fully in the stall and lying down associated behaviours, Chapter 3). These results from my observational studies may now form a basis for future, targeted research, such as on the environmental needs of ill cows.

5.2 Conclusions

My thesis contributes to the knowledge on sickness behaviours in dairy cows, specifically those diagnosed with fever and with metritis. The findings presented in this thesis also suggest that we know little about environmental preferences of sick cows and if common treatment strategies have beneficial effects for the cows. To better understand treatment effects in cows with diseases that cause malaise and pain, sickness and pain behaviours should both be considered.
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