THE ELIMINATIVE BEHAVIORS OF DAIRY CATTLE AND HOW THEY ARE AFFECTED BY OUTDOOR ACCESS IN TIE-STALL HOUSING

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ABSTRACT

Eliminative behaviors are the acts of defecation and urination and the behaviors surrounding these acts. Understanding the eliminative behaviors of dairy cattle is important for animal welfare and pertains to excreta management. As the dairy industry seeks to increase movement opportunity for animals, producers require more information about how cattle behavior will be affected. This thesis aimed to determine how outdoor access might affect the eliminative behaviors of tie-stall-housed dairy cows via a literature review and an experimental study.

The literature review revealed that there is little information regarding the eliminative behaviors of dairy cows, specifically. The eliminative behaviors of dairy cattle have been most thoroughly described in terms of frequencies/24 h and hourly distributions of these frequencies. There are relationships between eliminative behaviors, barn activities, and activity levels of the animals, which suggests that housing system plays a major role in this aspect of behavior. However, these studies vary by year, breed, and region, and recent studies on tied cows in a North American context do not exist. There is a gap in knowledge in the relationship between exercise/movement opportunity and eliminative behaviors of dairy cows.

The experimental study aimed to describe eliminative behaviors at tie-stalls, determine whether outdoor access affects daily patterns of elimination, and determine whether outdoor access duration affects elimination frequencies. Holstein cows were blocked by parity and days in milk such that Control cows (winter 7; summer 8), which remained inside at tie-stalls for the duration of the experiment, were paired with Outdoor cows (winter 8; summer 7), which were provided 5 d/wk (weekdays) outdoor access in paddocks for 1-2 h over 2-wks. Pedometers recorded step and lying activity continuously. All cows were video recorded for 48 consecutive h (weekends) with no outdoor access. Frequencies, durations, activity, position, where excreta fell, and whether the event occurred within 3 min of standing were continuously observed. Eliminations were recorded live concurrently for Outdoor cows throughout the duration of their time outdoors, and for Control cows during the first h of outdoor access (winter 3 d; summer 4 d). Eliminations of Outdoor cows allocated 2 h outdoor access were analyzed to compare the first vs. second h outdoors (winter 8 d; summer 9 d).

Elimination frequencies/24 h in tie-stalls were the following (AVG. \pm SD): defecations, winter: Control = 19.4 \pm 2.73, Outdoor = 19.1 \pm 1.58; summer: Control = 21.1 \pm 4.12, Outdoor =

18.6 ± 1.18; urinations, winter: Control = 11.4 ± 2.83, Outdoor = 11.0 ± 3.33; summer: Control = 9.2 ± 1.49, Outdoor = 9.4 ± 0.98. Graphical assessment showed that eliminations were most frequent during 5:00 milking and feeding and from 19:00-20:00; the latter reflected when the greatest numbers of steps/cow were taken. Neither times with the greatest proportions of elimination frequencies nor times of high step activity were consistently related to percentages of eliminations that fell into the stall. Elimination rates during 1 h outdoor access were not different from rates observed for Control cows (defecations and urinations; P > 0.05). For 2 h Outdoor cows, defecation rates during the first h outdoors were greater than during the second h (P > 0.05). Urination rates during the second h outdoors were greater than during the first h (0.55 vs. 0.28 events/cow/h; P < 0.05). Frequencies of defecations and urinations/cow were greater during the middle and end of outdoor periods, respectively. High numbers of steps/cow did not reflect increased elimination frequencies as they did during observations at tie-stalls, though cows during the summer took more steps outdoors compared to cows during the winter. Such information will provide insights to designing and managing housing systems for dairy cows which allow for partial outdoor access.

RÉSUMÉ

Les comportements d'élimination incluent la miction et la défécation. Comprendre leur expression chez les bovins laitiers est important dans un contexte où la mise en place de pratiques augmentant les opportunités de mouvement des vaches risque de changer la gestion des excréments à la ferme. L'objectif de ce mémoire était donc d'évaluer l'impact de l'accès à l'extérieur sur les comportements d'élimination des vaches laitières.

Très peu d'études sur ces comportements sont disponibles; la plupart les décrivent par des fréquences sur 24h, ou distribuées par heure, et identifiant des liens entre le logement et les éliminations. Toutefois, les études publiées diffèrent entre elles en ce qui a trait aux années, aux races utilisées, et aux régions; aucune étude récente n'a été conduite en stabulation entravée en Amérique du Nord. Il en reste donc à apprendre sur les liens unissant les opportunités de mouvement et les comportements d'élimination des vaches laitières.

Notre expérience visait à décrire les comportements d'élimination à la stalle chez les vaches laitières en stabulation entravée, à déterminer comment l'accès régulier à une cour d'exercice extérieure affecte la distribution journalière des éliminations, et s'il a un impact sur leur fréquence. À cette fin, des vaches Holstein précédemment mises en paires selon leur nombre de parités et de jours en lait ont été réparties entre deux traitements : les vaches témoin (7 en hiver, 8 en été) restaient à l'intérieur, à leur stalle en stabulation entravée pour la durée du projet, alors que l'autre groupe (8 vaches en hiver, 7 en été) avait un accès régulier à l'exercice, 1 ou 2h par jour, pour 2 semaines. Des podomètres ont enregistré en continu le nombre de pas et les données de repos. Toutes les vaches ont été filmées durant 48h consécutives sans accès à l'extérieur, pour observer la fréquence des éliminations, leur durée, l'activité durant laquelle elle se produisaient, l'endroit où les déjections tombaient et si l'élimination se produisait dans les 3 minutes suivant le lever. Pour les vaches avec accès à l'exercice, les éliminations ont été relevées par observation directe durant tout le temps passé dehors; pour les vaches à l'intérieur, elles l'ont été durant la première heure d'exercice (hiver: 3 jours; été: 4 jours). Les éliminations des vaches dehors pour 2 heures ont servi à comparer la première à la seconde heure (hiver: 8 jours; été: 9 jours).

En hiver, les défécations/24h durant la période d'exercice furent de $19,4 \pm 2,73$ pour les vaches à l'intérieur et de $19,1 \pm 1,58$ pour les vaches dehors. En été, elles furent de $21,1 \pm 4,12$ et $18,6 \pm 1,18$ défécations/24h respectivement pour ces deux traitements. Les urinations/24h furent

en hiver de 11,4 \pm 2,83 et 11,0 \pm 3,33 pour les vaches témoin et les vaches dehors, respectivement. En été, elles furent de 9,2 \pm 1,49 et 9,4 \pm 0,98 urinations/24h respectivement pour ces deux traitements. Les éliminations étaient plus fréquentes durant la traite matinale (05:00), les périodes où les vaches s'alimentaient, ainsi qu'entre 19:00 et 20:00, alors que le nombre de pas effectués par les vaches augmentait. Le pourcentage d'éliminations tombées à l'intérieur de la stalle n'était pas associé aux fréquences d'éliminations ni au nombre de pas plus élevés. Les taux d'éliminations de la première heure de la période d'exercice ne différaient pas entre les traitements (défécations et urinations; P > 0,05). Les taux d'urination étaient plus élevés durant la première que durant la seconde heure (0,55 vs 0,28 urinations/vache/h; P < 0,05). La fréquence des éliminations augmentait à la mi-temps et à la fin de la période d'exercice, mais n'était pas liée au nombre de pas.

En plus d'aider à brosser un portrait plus précis de la répartition temporelle des éliminations, les informations collectées durant ce projet serviront à la mise en place de systèmes de logement incluant un accès contrôlé à l'extérieur pour les vaches laitières.

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CONTRIBUTION OF AUTHORS

In this thesis, two multi-authored manuscripts are presented. The authors of both manuscripts 1 and 2, presented as Chapters 2 and 3, respectively, are:

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CHAPTER 1 – GENERAL INTRODUCTION

Housing systems for dairy cattle can be defined by the degree of movement that can be achieved by the animals (Shepley et al., 2020). On one end of the spectrum are tie-stall barns involving indoor systems in which animals are tethered to an individual stall for all or part of the day or year; on the other are pasture-based systems allowing animals to graze as weather and pasture quality permits. To note, this thesis labels tie-stall and stanchion barns separately in studies where specified, but both involve the least amount of movement due to confinement of the animal to a stall for all activities. Thus, when barn types are not specified, the term "tie-stall" is used and includes stanchion barns. The tie-stall provides all needs of the cow such that she is able to eat, drink, rest, and be milked without leaving her stall. Theoretically, it should allow the producer to closely monitor individual animals to provide care and carry out routine procedures such as breeding or keeping track of feed intake. The indoor aspect of tie-stall barns is also important for protection from the elements, particularly during the winter.

Per the latest statistics, approximately 73% of 6,138 recorded Canadian dairy farms were reported as tie-stall barns, with the remaining percentage consisting of free-stall barns; systems where cows are loose and have access to lying cubicles (CDIC, 2019). Tie-stall and free-stall barns represented approximately 39% and 40%, respectively, of the recorded dairies in the United States as of 2013; of which about 77% of the total dairy operations in the country were studied (USDA, 2016). However, the proportion of tie-stall barns worldwide is decreasing due to both a general increase in herd size and increasing levels of concern for animal welfare-related issues expressed by parties within and outside of the dairy industry (Barkema et al., 2015). Numerous studies exist comparing the welfare of dairy cattle housed in tie-stalls verses other housing systems. Some of the known poor welfare outcomes reported in tie-stall cows are related to physical health, such as high prevalence of leg injuries (e.g., Nash et al., 2016). Psychological health can be affected as shown via the possible associations between stereotypic behaviors, like tongue rolling and bar-biting, and tethering (e.g., Redbo, 1992). Additionally, as reviewed by Beaver et al. (2020), the general public believes that dairy cattle should be able to live "naturally" in the sense of having access to pasture (outdoor verses indoor housing) and being able to properly socialize (free-stall/pasture verses tie-stall housing).

Thus, there is increasing interest in providing spaces such as outdoor paddocks, yards, or even indoor facilities to increase movement opportunity for dairy cattle. Indeed, the revision of the Canadian Code of Practice for the Care and Handling of Dairy Cattle that began in 2019 lists "exercise" and "outdoor access (pasture and alternatives)" as "priority welfare issues" regarding indoor housing systems (NFACC, 2020). It will be important to better understand all aspects of dairy cattle behavior as the industry moves towards alternative methods of housing and management. One such aspect are eliminative behaviors; that is, the acts of defecation and urination and associated behaviors.

1.1 Hypothesis and Implications

Knowledge of the eliminative behaviors of dairy cattle is inherent for the proper management of excreta. However, there is limited information available in terms of outlining the typical behaviors for cows in tie-stall systems specifically. Additionally, there are gaps in knowledge regarding whether increased movement associated with outdoor access could affect the eliminative behaviors of tied cows. It was hypothesized that during days in which all cows were inside at tie-stalls, cows previously allocated to outdoor access would show similar eliminative behaviors to cows which remained inside at tie-stalls at all times. It was also predicted that during days in which cows were allocated to outdoor access, elimination rates for cows outdoors would be greater than for cows which remained inside at tie-stalls at all times. Finally, it was predicted that cows allocated to 2 hours of outdoor access would not show differences in elimination rates between the first and second hours of being outdoors.

This thesis consists of a literature review and an experimental study. The literature review examined the current available information regarding eliminative behaviors of dairy cattle to define the term and highlight trends that were observed, then described what factors can affect eliminative behaviors. It also detailed why studying this particular aspect of behavior is important for the welfare of the animals and for the dairy industry's shift away from continuous tie-stall housing. The subsequent study involved tie-stall housed lactating Holstein cows that were part of a larger experiment examining the effects of outdoor exercise access on behavior and activity. We first described eliminative behaviors during 48 consecutive hours of indoor video observation in relation to cow activity and activities of barn staff. Then the elimination rates and step activity of cows allocated to 1 or 2 hours of outdoor access were recorded to compare between cows not allocated to outdoor access and between the first and second hours of outdoor access.

1.2 Objectives

1.2.1 Overall objectives

The main objectives of this thesis were to review the literature regarding the eliminative behaviors of dairy cattle and to determine how outdoor access might affect the eliminative behaviors of tie-stall housed cows.

1.2.2 Specific objectives

More specifically, this thesis aimed to

- 1. define, describe, and emphasize gaps in knowledge regarding the eliminative behaviors of dairy cattle based on the available literature.
- 2. describe eliminative behaviors at tie-stalls.
- 3. determine whether outdoor access affects daily patterns of elimination.
- 4. determine whether duration of outdoor access affects elimination frequencies.

Such information will provide producers with management practices and options to fully implement movement opportunities for their animals.

CHAPTER 2 – THE ELIMINATIVE BEHAVIORS OF DAIRY CATTLE: A REVIEW

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2.1 Abstract

The eliminative behaviors of dairy cattle include frequencies and distribution over time and space for defecations and urinations, how the animal responds to internal and external factors by way of altered patterns of defecation and urination, and how an animal carries out and responds to its own acts of elimination. This review discusses the available literature in order to first define and describe eliminative behaviors, then outlines what is currently known about eliminative behaviors of dairy breeds specifically. What follows is a discussion on what can affect eliminative behaviors, the relationships between eliminative behaviors and welfare, and methods for managing eliminative behaviors and excreta. Information on eliminative behaviors is sparse for dairy cattle in tie-stall systems and is largely centered around frequencies and distributions over time per 24 hours. There are relationships between eliminative behaviors and activity levels of the animals and the people who manage them, suggesting that the types of housing systems play a key role in this aspect of behavior. For dairy cattle it also seems that individual animals vary in their eliminative behaviors, in which case it may be interesting to determine what aspects of their individuality contribute to these differences. The dairy industry's shift towards partial- or total-outdoor access systems introduces the question of excreta management. Further research should focus on refining what are typical eliminative behaviors for dairy cattle in certain housing types and regions, or of a specific breed; and determining how the eliminative behaviors of dairy cattle are affected by changes to housing and management routines associated with increasing exercise and movement opportunity.

2.2 Introduction

Eliminative behaviors include those surrounding defecation and urination. The eliminative behaviors of dairy cattle have been described (e.g., Hafez, 1969; Phillips, 2002) and studied (e.g., Brantas, 1968; Aland et al., 2002), but not to the same degree of detail as the eliminative behaviors of other animals such as dogs, cats, horses, pigs, and goats. Studying the eliminative behaviors of dairy cattle is imperative to the welfare of the animals themselves; for instance, a better understanding of how and when eliminations occur might help in improving cleaning routines to manage excreta, thereby maintaining foot and udder health. It also has implications in human health and environmental impacts, one example being the emission of ammonia, which is an air pollutant and contributes to eutrophication (Hristov et al., 2011).

The purpose of this literature review is to discuss the current available information related to the eliminative behaviors of dairy cattle, divided into several sections: defining and describing eliminative behavior, outlining what is known about eliminative behavior, describing what factors affect eliminative behavior, stating why knowledge of eliminative behavior. It closes important, and presenting methods for and attempts at controlling eliminative behaviors. It closes with the current status of the dairy industry, and the further significance eliminative behaviors will play in impending changes to management practices. This review aims to examine the literature surrounding dairy breeds specifically, but due to the limited information available, it makes some reference to cattle in general (i.e., beef breeds and *Bos indicus*/Zebu). "Eliminations" will be used to refer to defecations and urinations collectively, unless specified in certain studies.

2.3 What are eliminative behaviors?

2.3.1 Defining and describing eliminative behavior

An eliminative behavior is a "behavior associated with the elimination of feces and urine from the body" (Semantic Scholar, n.d.). Further descriptions of eliminative behavior vary slightly throughout the animal literature. For instance, this phrase is most often utilized in books where the purpose is to explain all aspects of behavior for a particular species. No actual definition is provided in most cases, but the writing may go into very specific details such as development of defecation and urination processes from birth. However, one equine behavior book states that "eliminative behaviors are used to get rid of accumulated body waste, and as is true in some other species, urination and defecation can also have a social function" (Beaver, 2019, p. 211). Research papers do not usually provide a definition either, and some only refer to observed frequencies of defecations and urinations in their usage of the phrase "eliminative behavior." It should be noted that information surrounding this topic is also labelled as "elimination behavior," though it carries the same meaning. Descriptions of the eliminative behaviors of dairy cattle have been published, but not to the level of detail seen in companion species or other production animal species. Following the most thorough definitions stated above and the research presently available, this literature review defines eliminative behavior in the context of dairy cattle to include three aspects: (1) frequencies and distribution over time and space for defecations and urinations, (2) how the animal responds to internal and external factors by way of altered patterns of defecation and urination, and (3) how an animal carries out and

responds to its own acts of elimination. Eliminative behaviors are an element of nutritional behavior and relate to body maintenance. Furthermore, they are considered an element of behavioral and physiological needs (Phillips, 2002).

Dairy cattle are large bovine ruminants (Hall, 2002). The logical first step would be to examine the eliminative behavior of feral cattle to get an idea of what is "normal" outside of any human-related factors. Though there are several established feral cattle herds, none have been studied specifically for eliminative behaviors. Caution must be taken when referring to studies on cattle in semi-wild or similarly labelled conditions as the animals are typically of beef or dual-purpose breeds. Studies on feral groups of dairy breeds specifically do not exist in the literature, with the exception of a single paper on Kerry cows in "semi-feral conditions," examining grazing patterns (Lianne et al., 2001). Eliminative behaviors in the most "naturalistic" setting involve animals grazed on rangelands, which again are usually associated with beef breeds and focus only on defecation frequencies and spatial distribution for the purposes of nutrient management. Extensive pasture systems offer the least restrictive environment for dairy breeds, and their relationship to eliminative behaviors will be further discussed later in this paper.

Defecation and urination are natural bodily functions, and animals have natural rhythms that they follow. Kilgour's review of 22 studies on overall behavior at pasture (2012) reveals that cattle in general are diurnal, meaning that most activities occur during the day; more specifically grazing activity peaks during dawn and dusk hours. It follows that resting and rumination occur at night. Outside of these typical activity patterns, it has been suggested that cattle are not selective about where and when they eliminate, nor are they are naturally aware of when they are about to eliminate (Hafez, 1969). It has been presumed that eliminations are not voluntarily controlled (Hafez, 1969), and that defecation specifically is a "largely involuntary" process in that other behaviors can be performed while defecating (Whistance et al., 2007, p. 22). Other ungulates such as the dik dik and pronghorn utilize manure and urine, respectively, to amplify their own scent (Phillips, 2002). Meanwhile all equids share the trait of scent marking the eliminations of conspecifics with their own feces and urine, and domesticated animals of this grouping usually choose to eliminate in specific areas of their paddocks or stalls (Beaver, 2019). Cattle do not seem to exhibit this latrine behavior of repeatedly eliminating in the same location (Whistance et al., 2009), nor do feces and urine appear to serve as a mark of territoriality as they do in related species. However, cows do communicate via their urine, apparently increasing the

frequency of urinations during oestrus to broadcast their status (Phillips, 2002). In perhaps a more involuntary form of communication, urine has also been observed to express negative emotional state. Boissy et al. (1998) found that heifers presented with urine from a stressed companion had a longer latency to feed in a novel environment compared to when presented with urine from a non-stressed companion; similarly, heifers had a longer latency to explore a novel object in a familiar setting if said object was sprayed with urine from a stressed companion compared to urine from a non-stressed companion. To the author's knowledge, studies attempting to describe pre-eliminative behaviors in cattle (e.g., sniffing to select an area before eliminating) do not exist.

The posture assumed during elimination includes an arched back and raised tail, with the hind legs placed apart. During urination, the back is noticeably more arched (Aland et al., 2002). Dairy cows at pasture most frequently move away from their feces right after defecating, whether that be apparently purposefully or just by coincidence (Whistance et al., 2011). However, studies on behaviors following urination have yet to be conducted. The long-standing notion is that cattle do not appear to mind bodily contact with their own feces and urine (Brantas, 1968), nor the excreta of their herd mates (Hafez 1969). This perception appears contradictory to cattle's strong aversion to grazing near manure, likely to avoid ingesting parasites (Michel, 1955), and their preference for clean and dry lying areas over those dirty with manure and those wet with water (Schütz et al., 2019). Both examples involve allowing cattle the opportunity to exhibit feces avoidance, which reasons that an animal paying no mind to manure or urine might be a result of habituation to an environment that consistently entails contact with excreta. *2.3.2 Discrepancies regarding the selective nature of defecation and urination*

Anecdotal accounts in two studies present some conflicting points regarding cattle's selectivity of elimination sites. Kilgour (1975) published a study which attempted to utilize the open-field test to determine the temperament of dairy cows. While eliminations as a result of being placed in the test arena were attributed to being nervous or skittish, and therefore of poorer temperament, the author notes that defecatory responses in particular may occur for the purpose of establishing territory or for giving a familiar scent to an unfamiliar setting. Fifteen out of the 50 total animals participated in "pawing shavings," a behavior that occurred after a cow sniffed the manure piles of their previously tested cohorts. These cows proceeded to defecate after pawing the shavings, suggesting that defecating in the arena "is not a haphazard activity"

(Kilgour et al., 1975, p. 622). Though these implications of territoriality are intriguing in that all animals were lactating Jersey cows (female), the experimental schedule that they were subjected to is not typical to that of any conventional dairy systems. The amounts of mixing of individuals and groups, in other words, may mean such observations are not applicable to cows in typical pasture or tie-stall housing situations where territorial or social conflicts are not prompted by the continuous entrance and exit of new individuals in a specific area.

Bond et al. (2012) conducted observations as part of a study involving the interaction between cattle and river habitats at two sites in England. At the site that was partially fenced, the authors stated that the cattle utilized fencing structures as guidelines when travelling towards the river, as scratching/grooming posts, and for defecating and urinating against. The investigative nature of the cattle was suggested to be the cause of these behaviors, which had such an effect as to double the amount of time spent in riparian location types (i.e., near the river and not wooded) when compared to the site that did not contain any fencing. The presence of the fencing was the only difference between the two sites. No further detail is given, so another explanation for eliminating against the fencing may be due to the animals' proximity to these structures as they walked to and from the river. The cattle showed "preferential defecation" in aquatic habitats (i.e., in or near the water of the river), spending 3.2% of the time defecating compared to 0.6% of the time defecating in other location types when viewed as a proportion to the total number of observations in that location (Bond et al., 2012, p. 64). However, all animals were Holstein bullocks and young (between 10-12 months of age), making the comparison to lactating cows more difficult.

2.4 What is known about eliminative behaviors in dairy cows?

2.4.1 Elimination frequencies of dairy cattle

Following the present review's definition of the eliminative behaviors of dairy cattle, frequencies and distributions over time will first be discussed. A comparison of the frequencies of eliminations for dairy cows over a 24-hour period are listed in Table 2-1. The selected articles were based on data collection conducted via live or video observation, as opposed to estimation by prediction and "after-the-fact" methods such as mapping excreta patches. Methodologies aside from live or video observations tend to be in the context of nutrient management under the term "excretion" for defecations and urinations. Few studies which cover an entire 24 hours of observations for elimination frequencies exist for dairy breeds. There is variation within these

studies, but such differences may be attributed to a combination of several factors. Perhaps the main points to conclude are that cows appeared to consistently defecate more frequently than they urinate in a 24-hour period, but the number of events varied greatly between studies.

Authors	Housing	Breed and	Region	AVG. freq.	AVG freq.	Observed
Villettaz Robichaud et al., 2011	Free-stall	Holstein	Canada	9.8 \pm 4.2 SD 15.4 \pm 4.3 SD	7.0 \pm 3.1 SD 9.3 \pm 2.8 SD	No; 23 h/day
Acatincăi et al., 2011	Tie-stall stanchion	Romanian Black and White	Romania	8.60 Winter 8.50 Summer	8.40 Winter 7.35 Summer	Yes
Whistance et al., 2011	Pasture	Holstein Friesian	United Kingdom	11		No; 6 h/day
Oudshoorn et al., 2008	Cubicle + time-limited grazing	Holstein Frisian	Denmark	10.5 ¹	6.5 ¹	No; 4, 6.5, and 9 h/day
Whistance et al., 2007	Cubicle or straw yard	Holstein- Friesian	United Kingdom	Straw yard 9.4 ² , 12.8 ³ Cubicle 10.0 ² , 10.5 ³		No; 6 h/day
Aland et al., 2002	Tie-stall stanchion ⁴	Swedish Red-White, Swedish Holstein	Sweden	16.1 ± 3.11 SD	8.95 ± 2.27 SD	Yes
White et al., 2001	Pasture	Holstein and Jersey	U.S.	Holstein 10.8 ± 0.5 Jersey 10.9 ± 0.5	Holstein 9.0 ± 0.6 Jersey 8.7 ± 0.6	Yes
Sahara et al., 1990	Stanchion	Holstein; dry ⁵	Japan	9.0 ± 2.5	4.3 ± 2.2	Yes
Fuller, 1928; data published in Aland et al., 2002	Tie-stall	Ayrshire, Holstein, Guernsey, Jersey; dry ⁶	U.S.	14.79 ± 3.63 SD	6.81 ± 2.63 SD	Yes

Table 2-1. Average frequencies of eliminations per cow per 24 hours for dairy breeds.

¹Averages of eliminations were calculated using the eliminations per hour of the three different time treatments

²Low-yield cows

³High-yield cows

⁴ Intermittent restricted feed

⁵ All animals dry stage

⁶ Involved some cows in dry stage

2.4.2 Activity levels and individuality of animals have relationships to eliminative behaviors

Some aspects of eliminative behaviors in cattle are associated with activity level. Dairy breeds in stanchion barns seem to show more eliminations during times when cows and humans

are most active, during the times just prior to milking and during feeding. It then follows that frequencies are lower during resting periods after milkings and during the evenings (Aland et al., 2002; Acatincăi et al., 2011). Stanchion-housed dry cows were reported to have eliminated more frequently during the day, from 8:00 to 20:00, compared to at night, from 20:00 to 8:00, but specific diurnal patterns were not described (Sahara et al., 1990). Specific patterns have been observed in pastured Japanese black cattle (beef breed), where animals at two sites during summer and autumn seasons over six years all eliminated at greater frequencies during times of activity and grazing compared to while inactive or ruminating (Hirata et al., 2011). Free-stall systems for dairy cows may differ in this activity-elimination pattern. Whistance et al. (2007) observed most defecations to occur while cows were in the cubicle alleys, not lying or resting, which appears to align with the patterns of the studies previously mentioned. But consideration must be given as to what classifies as being "active," as the authors suggest standing in the cubicle alleys as opposed to the feed alleys was a result of lower comfortability of the stalls. Had lying areas been of a more suitable quality, this idle time might have instead been used for lying. The other free-stall study on eliminative behaviors by Villettaz Robichaud et al. (2011) showed patterns of standing and time at the feeder consistent with diurnal rhythms, but these were not closely associated with frequencies of defecations and urinations. It was concluded that eliminations were distributed evenly across 24 hours. Knowing that elimination frequencies may change throughout a day means observational studies which look at a specific segment of time per 24 hours could be affected.

The other general observation for eliminative behaviors is that there seems to be individual variation between animals. Looking at the standard deviations provided by the papers in Table 2-1, averages for defecations have a wider spread than averages for urinations in indoor systems. Aland et al. (2002) found that the factor of individual cow had a significant effect on the numbers of eliminations per 24 hours (P < 0.001). For example, one cow may average nearly double the number of defecations (approximately 22 events per 24 hours) compared to another (approximately 12 events per 24 hours). Over the nine days of observations, individual cows varied in their frequencies of both defecations and urinations, but no significant differences were found. This appears to be the same situation for free-stall systems, as the ranges in numbers of eliminations per 24 hours (Villettaz Robichaud et al., 2011) supported those in the above tie-stall study. These variations in frequencies were also not related to cow characteristics such as days in

milk and feed and water intake. The authors suggest "that cows that defecate and urinate more frequently do so regularly" based on correlations between days ranging from 0.49 to 0.77 and 0.28 to 0.70 for defecation and urinations, respectively (Villettaz Robichaud et al., 2011, p. 4895). In contrast, the distributions of average eliminations for cows at pasture were remarkably similar. Defecations for lactating Holsteins and Jerseys only varied by 0.5 events (SE), while urinations varied by 0.6 events (SE) for both breeds. Observations were carried out for five 24-hour periods, but it should be noted that this data for average eliminations was for only eight animals (four Holstein and four Jersey) total (White et al., 2001).

2.4.3 Amounts of excreta produced by cattle

Studies including measured volume amounts of excreta for cattle from a behavioral perspective are rare. Per 24 hours, cattle are estimated to produce 40 to 50 kg of feces and 15 to 25 L of urine. This is equivalent to an average animal under normal conditions producing approximately 6% to 7% of its body weight in excreta (Acatincăi et al., 2011). Another description in this aspect is by Hirata et al. (2011), regarding pastured beef cattle. Manure weights were measured via portable balance for each defecation event, then calculated as hourly proportions of total weight and of total defecation events per 24 hours. Animals produced a greater amount of feces per hour during the daytime while active, and produced a greater amount of feces per event during inactive periods. This appeared to reflect the fluctuations in elimination frequencies over a 24-hour period, as mentioned previously. Unfortunately, data showing the actual weights were not presented, and measurements of urinations were not taken.

Even rarer are reports of the durations of elimination events, in which there is opportunity to be used as a proxy for estimating the amounts of excreta produced. The stanchioned dry cows of the study by Sahara et al. (1990) defecated and urinated for average durations of 7.6 ± 2.6 and 11.7 ± 5.5 seconds, respectively. In contrast to Hirata et al. (2011), defecation durations did not vary between hours of the day. Urinations, however, were of longer durations for events occurring from 0:00 to 8:00, likely due to decreased frequency of events during this time period (Sahara et al., 1990).

2.5 What affects eliminative behaviors?

2.5.1 Internal effects

Descriptions of the eliminations of dairy calves over time and space lie in a single study by Vaughan et al. (2014a). Female Holstein calves were observed at two time periods (averaged 32 and 61 days old) and, similar to dairy cows in stanchion barns (Aland et al., 2002; Acatincăi et al., 2011; Sahara et al., 1990) and beef cattle at pasture (Hirata et al., 2011), showed greater elimination frequency during the active daytime hours. The fact that the calves were group housed and managed via automatic feeders makes for a curious comparison between the patterns observed in other studies in various housing methods. Additionally, there were large differences in elimination frequencies between calves, as was seen in most studies involving mature animals mentioned previously. Of further interest are the effects of age. There was a significant low positive correlation with frequency of elimination prior to weaning, whereas after weaning, differences were attributed to visits to the water feeder. Overall, there was a significant increase in elimination frequency after weaning that the authors do not attribute to the shift to solid feeds. After weaning, calves also eliminated more frequently on the slatted flooring near the feeding areas compared to the sawdust resting area. This was possibly the result of a combination of more frequent visits to the feeders and decreases in resting time.

There may be differences in eliminative behaviors between heifers and cows. Cows showed significantly more defecations, but not urinations, than heifers in a stanchion barn (Aland et al., 2002). Hirata et al. (2011) did not perform statistical analyses in comparing their beef heifers and cows, but the graphs outlining elimination frequencies per 24 hours and over 24 hours do not appear much different between groups. In comparing primiparous and multiparous cows in tie-stalls, the former group had significantly higher (poorer) scores for fecal contamination of the lying area. Size in this case was likely a confounding factor as the authors note an average 100 kg difference in live weight between parity groups, meaning that younger animals had a greater range of movement within their stalls. The situation was the opposite in cubicles, where primiparous cows very seldom dirtied their lying areas. Whether it was the difference in the importance of lying or differences in body condition between the age groups remains unclear (Herlin et al., 1994). Within cows specifically, age does not appear to affect elimination frequencies. In the case of lactating cows in tie-stalls (Fuller study from 1928, data published in Aland et al., 2002) and free-stalls (Villettaz Robichaud et al., 2011), neither age nor parity, respectively, were correlated to differences in daily elimination frequencies. In another study, lactating cows in a rotationally-grazed system were monitored for urination behavior with mechanical sensors. Like other studies the cows varied in their daily frequencies, but this difference was not associated with age (Draganova et al., 2016).

There is some evidence that a cow's patterns may change as a result of life stage. For example, the first experiment by Villettaz Robichaud et al. (2011) showed a correlation between frequency of urination and days in milk. However all cows in this group were pregnant compared to no pregnancies in experiment 2, which aligns closer to the explanation of lactation stage (i.e., pregnancy) rather than the average and variability in days in milk between cows. Aland et al. (2002) calculated significantly more urinations and a tendency for more defecations in lactating cows compared to dry cows in the analyses of Fuller's study. There was no interaction between breed and lactating or dry status, and no significant effect of breed. The observational study by Sahara et al. (1990) gives a baseline for the eliminative behaviors of dry dairy cows. It was stated that "feeding conditions influence the frequency of defecation" based on assumed differences in moisture content of feed provided to their dry cows verses feed provided to lactating cows of comparable studies (Sahara et al., 1990, p. 250). Such results from dry cows may be due to differences in feed and energy requirements. However, it has not been determined whether it is the rate of feed intake, for example that assumed by higher liveweights and greater supplemental feeding provided to a group (Hirata et al., 2011), or qualities of the feed, such as fiber content (Acatincăi et al., 2011) that affect the frequencies of eliminations.

2.5.2 External effects

Several sources state that temperature (Fuller, 1928 via Aland et al., 2002; Hafez, 1969; Oudshoorn et al., 2008; Acatincăi et al., 2011) and humidity (Hafez, 1969; Acatincăi et al. 2011) are known to have an effect on elimination frequencies, but with little specification as to how or in what way. The perception may be that higher temperatures lead to less frequent defecations and more frequent urinations. These changes would reflect cattle's decrease and increase in feeding and drinking activity, respectively, in response to heat stress (Kadzere et al., 2002). Acatincăi et al. (2011) observed their stanchioned lactating cows during the winter and summer seasons, with temperatures averaging 8.1 and 28.4 degrees C, respectively. Defecation frequency per day was not statistically different between the seasons, while during the summer there were significantly fewer urinations (P < 0.001). In comparison, increased urination frequency during the summer (compared to autumn) was the case in at least one study's groups of beef heifers and cows. Yet the authors reasoned these results were not due to increased water intake; rather, any alleviation of heat stress was instead achieved by cattle moving to the forested environment. Temperatures also varied by approximately only 1 to 4 degrees on average between the seasons (Hirata et al., 2011). Additionally, White et al. (2001) noted how dairy cows at pasture tended to stay closer to the water tank as the temperature-humidity index rose. A greater amount of time spent in the area led to a greater concentration of urinations. The literature appears to suggest that, rather than affecting physiology, temperature more so influences the immediate movement of cattle when they have the ability to control their position in the environment. As a result, there are changes in the distribution of feces and urine patches.

In comparing housing systems, we begin to see that eliminative behaviors can be affected in ways beyond daily frequencies. However for calves, the only evidence of a relationship between housing and eliminations lies in frequencies. Holstein-beef breed crossed calves were housed singly or in groups of three starting at one week of age. It was found that grouped calves eliminated more frequently than individually housed calves and, though they were subjected to different feed types, were presumed to consume similar amounts of water. Following this, the authors believed the differences in frequencies were a result of social facilitation (Phillips, 2004). If their prediction is true, it may be important to consider the social effect of eliminative behavior for mature animals. If some cows were to start defecating, for example, does this cause other cows to start defecating? However, one would first need to discern whether peaks in elimination frequencies are only associated with daily patterns/natural rhythms in conjunction with feeding and resting behaviors.

Even within types of indoor housing systems, eliminative behaviors may vary. Herlin et al. (1994) compared the fecal contamination of tie-stalls, feeding cubicles (lying stalls with a feed table in front), and cubicles, listed in order of time most to least occupied by the cow. Cubicles and stalls were cleaned during two periods daily, directly after morning and afternoon contamination scores were recorded. For multiparous cows, tie-stalls had a greater percentage of contaminated stalls in the afternoon compared to the other systems (P < 0.05) whereas feeding cubicles had a lower percentage of contaminated stalls in the afternoon consider that tie-stall cows spend their entire day in the stall. With that being said, the authors reasoned that the cleanliness of feeding cubicles was a result of concentrates being delivered at a separate location from the forage at the feeding table/lying stall. There is also evidence that the structures of housing systems affect the feces avoidance element of eliminative behavior. Cows in a more restrictive cubicle system remained lying or resumed lying after defecation more frequently as opposed to walking away from their

own manure in a straw yard system. Behavioral sequences showing incidental (e.g., moving away from feces while performing another activity) and intentional (e.g., stopping a behavior in order to defecate) avoidance were more frequent in the straw yard system, and avoidance was not as affected by milk yield classification as it was in the cubicle system (Whistance et al., 2007). It has been hypothesized that consistent exposure to excreta "may cause habituation to its offensiveness" (Phillips, 2002, p. 150). Consistent exposure in this case was described as a feature associated with indoor, loose-housing systems, but in the sense that cattle become accustomed to the odor of excreta rather than physical contact with it. Housing systems without open areas bring up the question of excreta avoidance, in which case it could be interesting to differentiate the notion between the avoidance of an animal's own waste or to that of its herdmates.

Outdoor and pasture housing systems for dairy cattle exist on a spectrum of duration of access to pasture and the actual presence of pasture. When lactating cows were given timelimited pasture access, frequencies of eliminations per cow per hour of outdoor time remained similar through 4, 6.5, and 9-hour treatments. Averaged results from visual observations and GPS logging showed 0.36, 0.37, 0.38, and 0.29, 0.25, 0.25 average defecations and urinations per hour, respectively. Cows altered their activity and time budgets to maximize amounts of grazing time, but this still did not affect elimination frequency. Furthermore, feces and urine were evenly distributed throughout the pasture for all time treatments (Oudshoorn et al., 2008). Observations on lactating cows in a rotationally grazed pasture also resulted in a lack of hot spots for feces and urine, to an extent. The time spent in a location was highly correlated to the number of eliminations in that location, such that warmer seasons saw significantly more eliminations within 30 meters of the single water tank. Other seasons resulted in even distributions of excreta. Ultimately 84.7% of total manure and 84.1% of total urine was deposited during the 20 hours of pasture time and only 10% of the total pasture was covered by excreta over the year of the study, which the authors use as arguments in favor of maximizing outdoor grazing periods (White et al., 2001). Whistance et al. (2011, p. 74) noted that, in conducting their feces avoidance studies on cows at a cubicle system, a straw yard system, and at pasture, "cows at grass did not eliminate as promptly as did housed cows and they also changed their behavior more shortly after defecating." These observations might be a function of the comfortability of lying surfaces and the decreased space and movement opportunity associated with cubicle and indoor systems. To

the author's knowledge, studies involving eliminative behavior in dry lot systems do not exist. One might propose that dairy cows behave similarly as in indoor, loose-housed systems, but with the added factor of temperature extremes requiring heat stress management (Tresoldi et al., 2017) that affects their placement within the pens.

2.5.3 Exercise and activity

The amounts of exercise and activity cows achieve are a function of the housing environment and management, and can relate to the overall health of the animals. It is suggested that exercise may affect the gastrointestinal tract in ways such as circulating blood flow away from the intestines and stimulating colonic motility in humans (Peters et al., 2001). In dairy cattle, exercise is recognized as a general benefit to physical health; for instance, by reducing feeding-related disorders (e.g., Gustafson, 1993), improving claw health (e.g., Loberg et al., 2004), and improving physical fitness and metabolic health (e.g., Davidson and Beede, 2009). However, eliminative behaviors are not noted in such exercise-related research. Studies which focus on the relationships between exercise and movement opportunity, metabolism, and feed intake (e.g., Anderson et al., 1979; Chapinal et al., 2010) do not appear to record defecation and urination either, though they provide ideal opportunities.

There are anecdotal notes that suggest a relationship between exercise and eliminative behaviors. One example is the description of the "Bovine treadmill," which states that "digestive upsets" can be impacted, and explains that "walking movement massages the entire bovine digestive tract" (U.S. Patent No. 7,654,229, 2010). In another case, a circular exercise mechanism was created to subject heifers and dry cows in late gestation to a controlled exercise regimen. The goal was to conduct exercise trials on the notion that "the general condition, muscle tone, and functional efficiency of the digestive, cardiovascular, and respiratory systems of the dry cow decline before parturition" (Anderson et al., 1977 p. 1173). Future research involving exercise for dairy cattle, whether it be forced or offering varying levels of movement opportunity, should at minimum record elimination frequencies. Such information would be helpful in both confirming the statements above and determining the practicality of managing excreta in exercise areas.

2.5.4 General arousal and affective state

Dairy cows respond to negative experiences via changes in eliminative behavior. Monitoring defecations and urinations is a common method of determining response levels in animals, and the relationship between eliminations and negative states such as fear and anxiety (Forkman et al., 2007) is well studied. For dairy cows specifically, it has been documented that more eliminations occur when tie-stall cows were placed in an unfamiliar room for milking. These effects of visual and olfactory isolation from conspecifics were significantly reduced by brushing by a familiar human (Rushen et al., 2001). More frequent eliminations were also the case when tie-stall cows were subjected to an aversive handling treatment (struck on the head and muzzle) compared to a gentle handling treatment (offered hay/grass/concentrates, stroked, gently spoken to). Thirty-two total treatment repetitions took place over five total days. Eliminations did not occur after the first six aversive and six gentle treatments over the first two days of experimentation, which the authors attribute to adaptation once cows learned to discriminate between handlers (Munksgaard et al., 1997). Lanier et al. (2000) observed that elimination was less likely in commercial auction ring conditions by beef cattle of various breeds with poor temperament scores. The authors supposed that "these highly excitable cattle probably defecated before reaching the auction ring" when faced with an unfamiliar setting and uncertainty, mainly stimulus in the form of motion and sound (Lanier et al., 2000, p. 1471). In a similar vein, defecation was likely in response to being separated from the rest of the group when a cow was to enter an open-field testing arena. Defecation occurred before actually entering the arena in many cases (Kilgour, 1975). Another elimination-related note regarding this point is that cattle under stress, in addition to defecating more frequently, will "produce more liquid feces" (Phillips, 2002, p. 150).

On the other hand, eliminative responses to events of positive connotation are less clear. For instance, returning to Kilgour's open-field test (1975), researchers noted that a majority of cows appeared to explore their new temporary environment, while some even pranced, a term which they described as being "excited." Considering the activation of the autonomic nervous system, which does not necessarily differentiate between positive and negative triggering, one might propose that increased eliminations could happen as a result of a more generally "aroused" state. Villalba and Manteca (2019, p. 2) argue that "both aversive (e.g., fighting) and rewarding situations (e.g., play and mating) may elicit a similar physiological stress response," and that the degree of control an animal has over its situation and environment should be the factor determining whether an event is perceived as negative or positive. However, the authors' notions are based on grazing animals, and the opportunities for providing enrichment that presents beneficial challenges will likely be more limited for dairy cattle in total confinement housing systems.

2.6 Why is monitoring eliminative behaviors important?

2.6.1 Relationship between eliminative behaviors and physiological health

Studying the eliminative behaviors of dairy cattle is important in the context of a production animal system; the main point being health and welfare. Cook (2002) discussed how housing system and design affect dairy cow hygiene, udder health, and lameness. Overall, their conclusions were to emphasize the importance of cow cleanliness for limiting new mastitis infection and maintaining healthy foot condition, both likely a result of wet and manure-covered environments. Poor hygiene scores of the lower leg are associated with loose-housing systems whereas poor scores of the upper leg and flank are more so found in tied cows. Inadequately maintained open lot systems see poor scores in all areas. Unhygienic environments are risk factors of hoof health issues such as interdigital dermatitis and heel horn erosion (Bergsten, 2001). In some cases, these environments can exacerbate leg injuries originally associated with uncomfortable stall surfaces, leading to tarsal or carpal bursitis and cellulitis (Bergsten, 2001). Manure on the udder, whether transferred from the lower legs and tail (Cook, 2002) or from contact of the stall or lying surfaces themselves, can introduce pathogens to the teat end. Udder hygiene score has shown significant associations with the prevalence of intramammary environmental and contagious pathogens in lactating cows housed in free-stalls (epidemiological study; n = 8 farms; n = 1250 cows). Subclinical mastitis in this case was 1.5 times more likely to affect cows scored as dirty as compared to clean (Schreiner and Ruegg, 2003).

2.6.2 Discrepancies in the relationship between eliminative behaviors and physiological health

Logic dictates that clean cows and a clean environment equal healthy cows, but this relationship is not always straightforward. For example, one study on free-stall cows (epidemiological; n = 14 herds) examined the relationship between milk somatic cell count (SCC) as an indicator of udder infection, and factors such as hygiene, body condition score (BCS), and lying time (Zambelis et al., 2019). High SCC, categorized as $\geq 200,000$ cells/mL, was found to only be associated with low BCS when compared to normal BCS cows, and not with hygiene of the udder, upper legs/flank, nor the lower legs. The authors hypothesize this conundrum to be a result of interactions between greater milk yield and feeding and lying behavior because cows with low BCS were associated with poorer hygiene of the lower legs,

specifically (Zambelis et al., 2019). For studies involving hygiene scoring at a single point in time, it may be difficult to determine the course of events; the dirtiness of the animal is typically perceived to be the cause of the health problem, but the health problem might have caused behavioral changes which result in a dirtier animal.

An early study by Zehner et al. (1986) showed that, under laboratory conditions, sterilized samples of recycled dry manure, chopped straw, and fine hardwood chips supported growth for E. coli, K. pneumoniae, and S. uberis species. In comparison, chopped newspaper and softwood sawdust did not support bacterial growth to detectable numbers, presumably due to their lower nutrient content, greater dry matter content, and differences between the controlled and barn environments. The conclusions were that environmental mastitis can still pose a risk regardless of contamination of bedding with excreta, with moisture content being another concern for bacterial growth. More recently, an on-farm study (epidemiological; 26 tie-stall and 44 free-stall farms) analyzed the bacterial counts from unused (storage piles) and used (the back 1/3 of stalls) bedding (Robles et al., 2020). Within unused samples, recycled manure solids (RMS) showed the greatest counts of *Streptococcus* spp. compared to sand, straw, and wood products. Unused straw harbored gram-negative bacteria, particularly Klebsiella spp., to a higher degree than the other bedding types. Interestingly, used samples of RMS and sand showed greater percent dry matter compared to unused samples. This aspect of improved quality, however, did not appear to abate overall bacterial growth. The bacterial counts in used RMS, straw, sand, and wood products were increased by approximate factors of 6.4, 1.5, 2.9, and 2.4, respectively, compared to unused samples (Robles et al., 2020).

2.6.3 Relationship between eliminative behaviors and other animal welfare outcomes

However, eliminative behaviors still have key relationships with cow well-being. Lying time is one known indicator of cow comfort (Haley et al., 2000), which can be affected by elements of housing and, by extension, the deposition of excreta. In one case, O'Connor et al. (2019) examined the lying behavior of dry cows on and off woodchip stand-off pads, the practice of temporarily moving cattle to preserve pasture quality while keeping six-hour grazing periods per day. There was a decrease in lying time at the pad and an increase in lying time at the grazing paddock over the course of five weeks for groups whose pads were not refreshed with bedding, meaning that cows had attempted to compensate for lying time lost. Scoring of the appearance and "stickiness" of the pad surface, attributed to accumulated feces, urine, and rainwater,

determined that a moisture level above 75% was likely a factor in a cow's decision to lie down. As previously mentioned in the present review, dairy cows showed clear preferences for clean and dry resting areas over those dirty with manure and those wet with water. The cows subjected to the two unclean treatments without choice also experienced poorer quality of rest, as suggested by fewer observations of the body positions associated with sleep. The authors postulated that such observations are a result of a change in affective state. Furthermore, there was a tendency for cows to avoid urinating on the clean lying surface, implying perhaps conscious efforts to keep clean areas clean (Schütz et al. 2019). Therefore, eliminative behaviors must be considered because feces and urine play a crucial role in welfare.

2.7 How are eliminative behaviors and excreta managed?

2.7.1 Housing and management

Methods of controlling eliminative behaviors for dairy cattle begin with the housing system. Tie-stall and cubicle housing are designed to separate the animal from its excreta by use of brisket boards/neck rails to limit forward movement in the stall, curbs that keep the lying surfaces separated from excreta as it is scraped or flushed, and in some cases gutters directly behind stalls. Whether it be scraping and flushing alleys, cleaning out deep-bedded systems, or tilling compost bedded-pack, studies suggest a minimum of two maintenance periods per day (Bewley et al., 2017). The idea of a "hygienic relief area," essentially providing access to pasture or anywhere off of concrete, for loose-housed cows has also been suggested, because the physical grass and ground textures aid in cleansing the feet (Bergsten, 2001, p. 18).

Electric trainers are used to maintain cleanliness of the tie-stall. The devices deliver a shock if touched when the back is arched for defecation or urination and train the cow to step backwards for elimination (Phillips, 2002). A previous epidemiological study in Canada showed that trainers were in use in approximately 71.1% out of the 100 farms examined in Ontario and Quebec during 2011 (Charlton et al., 2016). One study (trial; n = 52 cows) showed significantly more manure in stanchions without trainers compared to those with trainers. Additionally, cows with trainers were 30% cleaner on the hocks and prevalence of heel-horn erosion was reduced over a three-month period (Bergsten and Pettersson, 1992). However, a later study examining tie-stall design in 317 dairies commonly found dirty hind limbs (up to/over the hock joint) and slightly dirty udders on cows with trainers installed. The use of trainers was observed in 76% of these Ontarian herds. Such discrepancies are likely due to improper placement of the trainers

(Zurbrigg et al., 2005). Ultimately, walking backwards is not a natural form of movement for cattle (Phillips, 2002). This fact in and of itself may contribute to the difficulties cows face in eliminating off of or beyond the stall curb, particularly for animals in tie-stall systems. Other factors to consider are the forward positioning of the cow while feeding, the tendency to defecate while feeding (Aland et al., 2002), and the natural process of cattle walking forwards while grazing (Phillips, 2002) or after defecating (Whistance et al., 2007; Whistance et al., 2011).

One straightforward concept of controlling eliminative behavior is to influence timing by way of management practice. Brantas (1968) described the training of cows to a cafeteria stable, a partial loose-housed system involving a bedded lying area and individual feeding stalls with headlocks. Following the tendency to eliminate after rising from resting and during standing periods, cows were to ideally eliminate into the excreta passage during four daily feeding sessions. Eliminations per cow per hour were to some degree greater during feeding (defecations: 0.68; urinations: 0.40) compared to during loose/resting periods (defecations: 0.52; urinations: 0.24). However, rates drastically rose (defecations: 2.92; urinations: 1.84) approximately halfway through the periods in-between sessions, consequently soiling the bedded area. Six feeding sessions might have resolved the issue, but this was deemed to be an impractical level of management. An experiment involving the use of footbaths was conducted on lactating cows to assess whether defecation can be stimulated. Neither treatments of walking through or standing in water-filled footbaths, nor having feet sprayed with water or air proved reliable in prompting this behavior. It appeared that the defecations observed were a result of novelty, as the total percentage of cows that defecated for control and any treatments combined reduced over the 26 days of experimentation (Villettaz Robichaud et al., 2013).

2.7.2 Training latrine behavior

Two attempts have been made to toilet train dairy cattle. The first study involved 14 to 16-month-old heifers trained in four phases: response to a clicker (classical conditioning), targeting a disc to reinforce the clicker, associating elimination with a reward on concrete (operant conditioning), and rewarding if elimination was on concrete. All heifers were successfully trained in the first three phases, and correct eliminations on concrete occurred nearly 2.5 times as often as incorrectly on straw. But, because concrete to straw movement prior to eliminating did not decrease as elimination on concrete increased, and straw to concrete movement prior to eliminating did not increase as elimination on straw decreased, actual toilet
training was not achieved. Heifers showed awareness of their own eliminations, but the incompletion of the final phase may have been due to the inability to associate flooring type with eliminations (Whistance et al., 2009). In the second study, separate sets of 1 to 2-month-old female calves were subjected to two experiments in an attempt to train urination to a specific location. The first calves were held in a stall and administered a diuretic to induce urination, then tested in the stall without diuretic (classical conditioning). The other calves were trained with diuretic in the stall then, during testing, received either a milk reward upon urination or a "time out" session if she failed to urinate within 15 minutes (operant conditioning). Operant-trained calves urinated more frequently in the stalls than their controls, while the opposite was the case for classically trained calves. On average, operant trained calves also urinated approximately twice as promptly as classically trained calves, but the former group had a large variation in how quickly they learned (Vaughan et al., 2014b).

Dirksen et al. (2020) state that a multitude of steps must be achieved in toilet training: recognizing impending elimination, retaining elimination, movement to the specified latrine, eliminating, leaving the latrine, and potentially associating this process with multiple latrine locations. Firstly, training regarding eliminative behaviors might be most successful with very young animals. Calves show evidence of stimulated elimination via the mother licking the perineal region and, by the third day of life, this reflex appears to be replaced by voluntary control. The authors also suggest that operant conditioning methods may prove superior in toilet training processes because defecation and urination involve both involuntary and voluntary muscle control. Dairy cattle have the capacity to be toilet trained under the correct conditions, but the practicality of managing these processes for industry settings should be considered.

One technological advancement towards toilet training is the "CowToilet" urinal. This system consists of a separate stall, which cows in loose-housing systems enter voluntarily to receive feed. Sensors determine the placement of the urine collection cup, which moves to stimulate the nerve superior to the suspensory ligament of the udder and activates the urinary reflex. Urine is collected separately from manure and can be stored for future use such as fertilizer or a source of hydrogen for energy. Preventing the mixing of manure and urine also reduces ammonia emissions, thus providing an improved environment for animals and barn staff. Currently there is not published evidence of this system's effectiveness as it is expected to be commercially available mid-2020 (Hanskamp, n.d.). Manual stimulation of the escutcheon nerve

is a well-known technique and is recognized in the literature for the collection of urine samples (e.g., Andersson & Larsson, 1952; LeBlanc et al., 2005; Løvendahl and Sehested, 2016). However, it does not seem to be referred to in textbooks or research specifically relating to eliminative behaviors.

2.8 Eliminative behaviors and dairy systems

Information about the eliminative behaviors of dairy cattle, including how these behaviors can be controlled and managed, will become increasingly relevant in addressing changes to the dairy industry. Housing systems with a focus on welfare and the expression of natural behavior are predicted to be the future of dairy housing. Such changes mean the phasingout of total tie-stall and total confinement systems (Bewley et al., 2017). There exists an extensive body of literature describing the negative effects of restrictive housing systems on the physiological and psychological well-being of dairy cattle, while also recognizing that indoor housing is beneficial in some respects (e.g., von Keyserlingk et al., 2009; Phillips et al., 2013; Arnott et al., 2017). One study out of Romania studying 80 tie-stall herds is particularly relevant for the present review, in that the authors compare systems utilizing year-long tethering and systems allowing *partial* outdoor access to paddocks and/or pasture. Nearly all scores for welfare criteria measures, such as lameness, ease of lying, and body language scoring, were better in outdoor access herds. None of the herds were classified as "excellent" for welfare quality, but 60% and 40% of outdoor access herds scored as "acceptable" and "enhanced," respectively. Of no-access herds, 35% and 65% scored as "not classified" (the lowest tier/failed the assessment) and "acceptable," respectively (Popescu et al., 2013). The general public's concerns also influence this shift towards more "naturalistic" systems. One review reflects on the various experiments involving free-choice and other types of outdoor access. The authors note that the public is open to these partial access methods as an alternative to 100% pasture time once they understand the reasons behind them (Weary and von Keyserlingk, 2017).

If producers are to transition to partial or total outdoor or unconfined systems, we need to know how this routine might affect eliminative behaviors. It also brings up the question of how excreta will be managed. In the case of the current Canadian Code of Practice for the Care and Handling of Dairy Cattle, the use of outdoor access/pastures/yards is recommended for providing an opportunity for exercise, for decreasing labor and bedding requirements for bedded- or composted-pack systems, and it is cautioned to prevent digestive problems by using gradual

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adjustment to pasture feeding (NFAC, 2009). Each situation, however, comes with its own problems: the specific relationships between exercise and eliminative behaviors are still unclear, access to outdoor spaces simply shifts the excreta and management problem from indoor to outdoor areas, and eliminative behaviors during the transition period to pasture are not outlined. Given that indoor systems remain some of the most common types of housing (Bewley et al., 2017), producers would benefit from protocols specifically dealing with the movement of cows into and out of the barn.

2.9 Conclusion

There is little information regarding the eliminative behaviors of dairy cattle. Of what does exist, most focus is on frequency and distribution over time and space per 24-hour period. Even within this information there are variances due to numerous factors specific to each situation, such as an animal's life stage or the amount of available space for movement. Eliminative behaviors from the animal perspective (i.e., natural history and behavior) are currently less clear, so the focus for industry settings should align with what *is* known to affect defecation and urination: housing type and human-animal interactions. The dairy industry would largely benefit from more research in pre- and post-eliminative behaviors, as this information will improve the management of excreta for remaining tie-stall and indoor housing systems. Ultimately, the transitional period as producers shift towards partial- and time-limited outdoor access systems leaves an array of questions surrounding eliminative behaviors, the topic of which already suffers from knowledge gaps.

2.10 References

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Connecting Text

The available literature surrounding eliminative behaviors of dairy cattle was reviewed in Chapter 2. In research papers and textbooks, the definition of "eliminative behavior" varies from simply frequencies of defecations to including the entire suite of behaviors pre- and postdefecation/urination. It was determined that most knowledge of tie-stall cows revolves around frequencies and hourly distributions of eliminations per 24 hours. Housing systems affect eliminative behaviors by influencing the degrees of movement cows can achieve, as well as the involvement and activities of the barn staff. However, there is little information regarding how increased exercise and movement opportunity might affect eliminative behaviors.

Chapter 3 describes a study that involved tie-stall housed lactating Holstein cows that were part of a larger experiment examining the effects of outdoor exercise access on behavior and activity. Cows will be separated into Control and Outdoor groups, denoting cows which remained inside at the tie-stall for the duration of the study and cows which were allocated to 1 or 2 hours of outdoor access, respectively. Cow step and lying activity will be continuously recorded via pedometers. Factors such as elimination frequencies, where excreta fell, and hourly numbers of steps will be recorded for 48 hours when all cows remain indoors at tie-stalls. On days when cows are provided outdoor access, eliminations will be recorded concurrently to compare rates of Control and Outdoor cows. Eliminations of cows allocated 2 hours of outdoor access will also be analyzed to determine whether duration affects rates. The results of this study will provide more information regarding the typical eliminative behaviors for dairy cows at tie-stalls on an hourly basis and determine whether outdoor access affects the eliminative behaviors of cows normally housed in tie-stalls. Both points are imperative for impending changes in housing and management practices associated with increased movement opportunity.

CHAPTER 3 – THE ELIMINATIVE BEHAVIORS OF TIE-STALL-HOUSED DAIRY COWS DURING WINTER AND SUMMER WITH AND WITHOUT OUTDOOR ACCESS

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3.1 Abstract

There is little information surrounding the eliminative behaviors of dairy cattle, particularly in the context of North American tie-stall systems. The introduction of alternative management and housing systems to allow increased movement opportunity also brings up the question of how excreta will be managed. This study aimed to (1) describe eliminative behaviors at tie-stalls, (2) determine whether outdoor access affects daily patterns of elimination, and (3) determine whether duration of outdoor access affects elimination frequencies. Holstein cows were blocked by parity and days in milk such that "Control" cows (winter: 7 cows; summer: 8 cows), which remained inside at the tie-stall for the duration of the experiment, were paired with "Outdoor" cows (winter: 8 cows; summer: 7 cows), which were provided 5 d/wk (weekdays) outdoor access in paddocks for 1-2 h over an 8-wk period. Pedometers recorded step and lying activity continuously on a per-minute basis. The following data collection focused on wk 7-8. For objective 1, all cows were video recorded for 48 consecutive h (weekends) with no outdoor access. For each elimination event, frequencies, times, durations, activity and position during, where excreta fell, and whether the event occurred within 3 min of standing were continuously observed. For objective 2, elimination events were recorded live concurrently for Outdoor cows throughout the duration of their time in the paddocks, and for Control cows during the first h of outdoor access (winter: 3 d; summer: 4 d). Data were analyzed using a mixed model with access type (Control or Outdoor), season, block, day, and the interaction between access type and paddock size as fixed effects, day as a repeated measure, and cow as a random effect. For objective 3, the elimination frequencies of Outdoor cows allocated to 2 h outdoor access were analyzed to compare the first and second hours outdoors (winter: 8 d; summer: 9 d). Data were analyzed using a mixed model with paddock size, observation hour (first or second), block, and day as fixed effects. The model was made into a nested model such that day within season, block, paddock size, cow, and period was a random effect and observation hour was a crossclassified fixed effect. The observations of eliminative behavior/24 h in tie-stalls showed the following (AVG. \pm SD): defecation frequencies, winter: Control = 19.4 ± 2.73 , Outdoor = 19.1 \pm 1.58; summer: Control = 21.1 \pm 4.12, Outdoor = 18.6 \pm 1.18; urination frequencies, winter: Control = 11.4 ± 2.83 , Outdoor = 11.0 ± 3.33 ; summer: Control = 9.2 ± 1.49 , Outdoor = $9.4 \pm$ 0.98; defecation durations (s), winter: Control = 12.5 ± 2.14 , Outdoor = 12.2 ± 2.52 ; summer: Control = 13.7 ± 2.74 , Outdoor = 11.8 ± 2.46 ; urination durations, winter: Control = 12.9 ± 2.99 , Outdoor = 13.6 ± 2.40 ; summer: Control = 15.5 ± 3.37 , Outdoor = 14.1 ± 1.80 . Graphical assessment revealed that elimination frequencies were most frequent during 5:00 milking and feeding and from 19:00-20:00. The evening peak reflected times when greatest numbers of steps/cow were taken. Neither the times with the greatest proportions of elimination frequencies nor the times of high step activity appeared to be related to hourly percentages of eliminations that fell into the stall. Elimination rates during 1 h of outdoor access were not significantly different from rates observed concurrently for Control cows (defecations and urinations; P >0.05). For Outdoor cows allocated 2 h, defecation rates during the first h outdoors were not significantly different from rates during the second h (P > 0.05). Urination rates during the second h outdoors were significantly greater compared to during the first h (0.55 vs. 0.28 events/cow/h; P < 0.05). Frequencies of defecations and urinations/cow were greater during the middle and end of outdoor periods, respectively. Numbers of steps/cow did not reflect increased elimination frequencies as they did during observations at the tie-stall, though overall, cows during the summer took more steps outdoors compared to cows during the winter. Information regarding potential relationships between eliminative behaviors, cow activity, barn schedules, and season can help in understanding the logistics of maintaining partial outdoor access systems.

3.2 Introduction

Eliminative behaviors are those associated with defecation and urination. Most of the information available for the eliminative behaviors of dairy cattle involves the frequencies of defecations and urinations per 24 hours, and the hourly fluctuations of these frequencies. In the few studies where observations were conducted for full 24-hour periods, average elimination frequencies for lactating cows ranged from 8.50 to 16.1 defecations and 7.35 to 9.0 urinations. These studies vary in terms of year, housing system, breed, and geographical region, though numerically all cows defecated more frequently than they urinated (White et al., 2001; Aland et al., 2002; Fuller study from 1928, data published in Aland et al., 2002; Acatincăi et al., 2011). It appears that more eliminations occur when humans, and therefore cows, are most active in stanchion barns (Aland et al., 2002; Acatincăi et al., 2011). At pasture, beef cattle also show diurnal patterns such that eliminations are not uniformly distributed over time (Hirata et al., 2011). In any case it is important to consider that the spatial distributions of a cow's eliminations are of course dependent on how much time she spends in an area (e.g., White et al., 2001).

However, there is little information surrounding how exercise and movement opportunity might affect eliminative behaviors; particularly for animals originally accustomed to tie-stall housing.

Research pertaining to eliminative behaviors is important for the welfare of dairy cattle. The presence of excreta in the environment is known to negatively impact foot health (e.g., Hultgren and Bergsten, 2001), while manure on the udder and lower hind limbs has been associated with elevated somatic cell scores which increases the risk of mastitis (e.g., Reneau et al., 2005). Dairy cows have also shown avoidance of feces in ways such as a tendency to stand for defecation or to walk away after defecating (Whistance et al., 2007), and a preference for manure-free and dry lying areas (Schütz et al., 2019). As a result, previous studies have aimed to determine times when cleaning the barn or stalls might be most effective (e.g., Aland et al., 2002; Villettaz Robichaud et al., 2011). Excreta management is also relevant for human health; for instance, the control of ammonia emissions as an air pollutant and as a contributing factor to eutrophication (Hristov et al., 2010).

Furthermore, the dairy industry is likely to shift away from year-long or continuous tiestall housing systems to include increased movement opportunity for the animals in the form of paddocks, yards, and potentially indoor exercise areas (Bewley et al., 2017). This is because epidemiological studies have reported poorer welfare outcomes (such as lameness or injury prevalence) in tie-stalls versus systems which provide some outdoor access, especially pasture (e.g., as reviewed in von Keyserlingk et al., 2009; Arnott et al., 2017). The general public also believes that dairy cattle should be allowed pasture access such that natural behaviors can be expressed (Beaver et al., 2020). Thus, the objectives of this study were to (1) describe eliminative behaviors at tie-stalls, (2) determine whether outdoor access affects daily patterns of elimination, and (3) determine whether duration of outdoor access affects elimination frequencies. Such information will prove valuable regarding the practical aspects of keeping cows and their environment clean in the event of changes to housing and management practices.

3.3 Materials and Methods

3.3.1 Ethics statement

The use of animals and all procedures were approved by the Animal Care Committee of McGill University and affiliated hospitals and research institutes (protocol #2016-7794).

3.3.2 General experimental design

Cows were part of a larger study examining the effects of outdoor exercise access on behavior and activity and were blocked by parity and days in milk. "Outdoor" cows were put through 8 different treatment rotations of paddock size and time outside in the design of an 8x8 Latin square. They were paired with their "Control" cow, which remained inside at the tie-stall for the duration of the experiment. "Companion" cows experienced the same routines as their Outdoor partners such that cows in paddocks were in pairs. Data from Companion cows was not collected. Outdoor cows were put through their treatments 5 days per week, then rotated to the next treatment the following week. This entire operation was repeated during the winter (early February through early April 2019) and summer (early July through end of August 2019) seasons. The Outdoor and Control cows enrolled in the winter were not enrolled in the summer repetition.

3.3.3 Animals

All animals were lactating Holstein cows. Winter trial consisted of 16 cows originally enrolled, with 1 Control cow removed due to injury to the udder. Summer trial consisted of 16 cows originally enrolled, with 1 Outdoor cow removed due to lameness. Descriptions are listed as of enrollment at the beginning of the larger experiment (AVG. \pm SD): winter, parity = 2.0 \pm 1.46, body condition score (BCS) = 2.7 \pm 0.43, daily milk production (kg) = 36.1 \pm 9.96, days in milk (DIM) = 148.9 \pm 63.46, hip height (cm) = 155.4 \pm 2.18, hook bone width (cm) = 63.4 \pm 3.93; summer parity = 2.5 \pm 1.68, BCS = 2.6 \pm 0.34, daily milk production (kg) = 47.1 \pm 11.42, DIM = 111.8 \pm 63.45, hip height (cm) = 155.8 \pm 3.72, hook bone width (cm) = 65.5 \pm 3.90. *3.3.4 Management*

The experiment took place at McGill University's Macdonald Campus Dairy Complex (Quebec, Canada). Enrolled cows were housed in 2 tie-stall rows, 12 cows per row, with blocks of cows in neighboring stalls. The average tie-stall length was 188.3 cm, and chain length was 1.00 m for all stalls. The gutters behind the stalls were covered with a grid. The stall bedding consisted of KKM longline rubber mats (Gummiwerk Kraiburg Elastik GmbH & Co. KG, Tittmoning, Germany) plus an approximate 2 cm layer of sawdust bedding. Fresh bedding was added in the mornings and in the afternoons (winter) and evenings (summer) for those cows which were considered by barn staff to need it.

Artificial lighting was turned on at approximately 5:00 and off at 20:30 (winter) and 21:00 (summer). Cows were milked twice daily in their stalls for both seasons at 5:30 and 17:00. The barn's scheduled feeding times were 6:00 and 10:00 for both seasons, then 13:00 and 19:00 (winter) and 12:00 and 15:45 (summer). Ration components were as follows, with concurrent variation between cows based on body condition, DIM, milk production, and when cows were to be dried off: winter high production group = 78.87% fibers and 21.13% concentrates, low production group = 82.25% fibers and 17.75% concentrates; summer high production group = 84.38% fibers and 15.62% concentrates, low production group = 90.51% fibers and 9.49% concentrates.

3.3.5 Present study

3.3.5.1 General experimental procedure

The present study focused on the last 2 weeks of the original experiment (winter: March 24 through April 5, 2019; summer: August 10 through 23, 2019). Outdoor conditions consisted of paddocks utilizing electric fencing. Neither water nor feed was provided during outdoor access, and ground cover was wood chips (winter) and grass (summer). The weekday routine consisted of 5 days of outdoor access periods of 1 or 2-hour durations, of which the daily procedure is illustrated in Figure 3-1. On the weekends, all cows remained indoors at the tie-stall. 3.3.5.2 Indoor observation procedure

Overhead video recordings (Smart Turret 2.8, Hikvision Digital Technology Co., Ltd., Hangzhou, China) were taken on 2 consecutive days during which no cows went outside, resulting in 48 hours of continuous video for each season. The same procedure was followed for winter and summer. Video was recorded on the weekend before week 7 of the larger trial. These video recordings were analyzed by one observer to assess the cows' eliminative behaviors, conducted via continuous data collection. For each elimination event, the time (hour, minute, second), duration (per second), position and activity during elimination, where excreta fell, and whether the elimination was within 3 minutes of standing were recorded. The ethogram is shown in Table 3-1 and was adapted from Zambelis et al. (2019). The times each cow stood from lying were recorded, and standing was defined as the time once all legs were straight from a lying position (Lidfors, 1989). During this video observation, barn activities of the times each cow was milked, the times each stall was scraped (cleaned), the times feed was delivered, and the times fresh bedding was added were all recorded. During the winter, indoor climate conditions were not recorded. The temperature and relative humidity for outdoor conditions on these days were retrieved from recordings from the nearest weather station and averaged -3.8 C and 45.3%, respectively (Government of Canada, 2019a; Government of Canada, 2019b). During the summer, the temperature and relative humidity for indoor conditions were retrieved from data loggers mounted to the stall dividers and averaged 21.0 C and 67.5%, respectively (HOBO Onset MX2305, Onset Computer Corp., Pocasset, MA, USA).

3.3.5.3 Outdoor observation procedure

Live, continuous observations for Outdoor cows began once the first cow left the barn and continued until the last cow entered the barn again. Data was collected by 3 observers. These observations took place during weeks 7 and 8 of the original experiment; 8 days for the winter and 9 days for the summer. Live, continuous observations for Control cows, in the tie-stall barn, were taken concurrently during the first hour of Outdoor access (Figure 3-1). These observations also took place during weeks 7 and 8 of the original experiment but for 3 days during the winter and 4 days during the summer, by 1 observer. Data collection for both seasons consisted of the time and type of elimination for each event.

During the winter, temperature and wind speed were measured outdoors prior to cows exiting the barn, with an average wind chill temperature of -3.1 C (Thermo-Anemometer, Extech 45158 Mini, Extech Instruments, Inc. via FLIR Systems, Inc., Wilsonville, OR, USA). During the summer, the temperature and relative humidity for outdoor conditions were retrieved from data loggers mounted near the paddocks and averaged 20.6 C and 72.6%, respectively (HOBO Onset MX2305, Onset Computer Corp., Pocasset, MA, USA).

3.3.6 IceTag and manure measurements

IceTag 3D pedometers (IceRobotics Ltd, Edinburg, Scotland, UK) were attached to the hind legs of all cows to record the numbers of steps and durations of lying bouts on a per-minute, continuous basis. The application of these pedometers to tie-stall housed cows has previously been validated (Shepley et al., 2017).

Manure was collectively weighed and volume measured on the same days that live, indoor observations were conducted. Collection was done via wheelbarrow and shovel. Rubber mats were placed over gutters for Control cows, and for Outdoor cows, observers waited until all cows were back inside the barn to collect manure. Manure was weighed (Weigh-Tronix Class III WI-125; sold by Staveley Weighing & Systems Canada inc. and manufactured by Weigh-Tronix inc., Fairmont, MN, USA; serial number 018 351) in the wheelbarrow and then volume measured with buckets. Measurements will be presented on a weight-per-event and volume-per-event basis for Control cows and for Outdoor cows provided 1 hour of outdoor access.

3.3.7 Statistical analyses

<u>3.3.7.1 Objective 1 – describe eliminative behaviors at tie-stalls</u>

Elimination frequencies and durations, minimums, and maximums per 24 hours for all cows were calculated and averaged over the 2 observation days. Information was shown averaged as categories by season and access type (Control or Outdoor cow). The percentages out of total eliminations (all cows collectively) per hour of a 24-hour period were calculated. These hourly elimination percentages were averaged over the 2 observation days and, together with barn management activities over a 24-hour period, were assessed graphically. The numbers of steps and durations of lying time per cow were averaged over the 2 observation days and assessed graphically, in a similar manner. Information was shown as separate seasons for both accounts. The percentages of occurrences out of total eliminations for categorical data of position and activity during elimination, where excreta fell, and whether the elimination was within 3 minutes of standing, were calculated and averaged over the 2 observation days. Information was shown averaged as categories by season and access type. The percentages of eliminations falling completely and partially in the stall out of total eliminations (all cows collectively) per hour of a 24-hour period were calculated, averaged over the 2 observation days, and assessed graphically. All calculations for descriptive data described above were completed using Microsoft Excel (Microsoft Corp., Redmond, WA).

3.3.7.2 Objective 2 - determine whether outdoor access affects daily patterns of elimination

All statistical models began by considering the 8x8 Latin square design of the original experiment. All statistical models were analyzed in SAS 9.4 (SAS Institute Inc., 2012, Cary, NC, USA). The original Latin square mixed model is shown below:

$$Y_{ijkm} = \mu + size_i + time_j + cow_k + period_m + (size * time)_{ij} + e_{ijkm}$$

Where: Y_{ijkm} is the dependent variable, the outcome measure of the k^{th} cow in the treatment of the i^{th} paddock size and the j^{th} time, during the m^{th} period; $size_i$ is the fixed effect of the i^{th} paddock size ($i = 20, 40, 60, \text{ or } 80 \text{ m}^2$); $time_j$ is the fixed effect of the j^{th} time (j = 1 or 2 hours of outdoor

access); cow_k is the random effect of the k^{th} cow; $period_m$ is the fixed effect of the m^{th} period (m = 1, 2, 3, 4, 5, 6, 7, or 8); $(size*time)_{ij}$ is the fixed effect of the interaction of the specific combination of size and time; and e_{ijkm} is the random error. To address the objectives of the present study, the following adapted models utilize the rates of eliminations per hour as the dependent variable. Rates of eliminations were calculated by dividing the frequencies of eliminations by the duration (in terms of hour) of observation. Each adapted model was run to analyze defecations and urinations separately. Data from one Control cow and one Outdoor cow were missing for winter and summer seasons, respectively, and only periods 7 and 8 were included as part of the present study.

To compare elimination rates during 1 hour of observation of Control cows to those of Outdoor cows provided 1 hour of outdoor access, the effects of season, block, day, and access type were added to the original model:

$$Y_{ijkmnprs} = \mu + size_i + time_j + cow_{pk} + period_m + (size * time)_{ij} + season_n + block_{np} + day_{mr} + access_s + e_{ijkmnprs}$$

Where: cow_{pk} is the random effect of the k^{th} cow within the p^{th} block (p = 1, 2, 3, 4, 5, 6, 7, or 8); season_n is the fixed effect of the n^{th} season (n = winter or summer); $block_{np}$ is the fixed effect of the p^{th} block within the n^{th} season; day_{mr} is the fixed effect of the r^{th} day (r = Monday, Wednesday, Thursday, or Friday) within the m^{th} period (m = 7 or 8); and $access_s$ is the fixed effect of the s^{th} access type (s = Control or Outdoor).

The variable *NewGroup* was created to remove confounding of *access* and *time* such that NewGroup 1 = Outdoor, time 1 hour cows; NewGroup 2 = Outdoor, time 2 hour cows; and NewGroup 3 = Control, time 1 hour cows. *size* was removed from the model because it was not estimable; size 1 was only in NewGroup 3 whereas sizes 20, 40, 60, and 80 were found in both NewGroup 1 and NewGroup 2. Furthermore, *size* as a main effect no longer contributed to the model due to being accounted for in the *NewGroup*size* interaction. *period* was removed from the model because it was not estimable; the same days were not observed between the two periods. Furthermore, *period* as a main effect no longer contributed to the model, due to being accounted for via nesting of day_{mr} . The finalized model for this specific question is shown below:

$Y_{ikmnprw} = \mu + NewGroup_w + season_n + block_{np} + day_{mr} + (NewGroup * size)_{wi} + cow_{pniwk} + e_{ikmnprw}$

Where: $Y_{iknnnprw}$ is the dependent variable, the outcome measure of the k^{th} cow in the treatment of the i^{th} paddock size, during the m^{th} period, the n^{th} season, of the p^{th} block, the r^{th} day, and of the w^{th} NewGroup; *NewGroup_w* is the fixed effect of the w^{th} NewGroup (w = 1 or 3); *season_n* is the fixed effect of the n^{th} season; *block_{np}* is the fixed effect of the p^{th} block within the n^{th} season; *day_{nr}* is the fixed effect of the r^{th} day within the m^{th} period; (*NewGroup*size*)_{wi} is the fixed effect of the interaction of the specific combination of NewGroup and size; *cow_{pniwk}* is the random effect of the k^{th} cow within the p^{th} block, the nth season, the ith size, and the wth *NewGroup*; and $e_{ikmnprw}$ is the random error. Only Outdoor cows provided 1 hour of outdoor access were retained for addressing this question, so NewGroup 2 was deleted to remove all Outdoor cows provided 2 hours of outdoor access. Observations for urinations for Control cows during the winter were missing, but the model was run in the same manner. Please refer to Appendix 1 and Appendix 2 for more information about covariance structures, variance parameters, and coefficients of variance for rates of eliminations pertaining to this model.

<u>3.3.7.3 Objective 3 – determine whether duration of outdoor access affects elimination</u> <u>frequencies</u>

The frequencies of eliminations per cow per 30 minutes over the duration of outdoor access were calculated. Total elimination frequencies were divided by the number of cows observed per 30 minutes of outdoor access, to account for the provision of either 1 or 2 hours of outdoor access. Boxplots were created to show the spread of frequencies over all days of the 2 weeks, as preliminary graphs did not illustrate clear differences between different days of the week. Information was shown separated by elimination type and season. The numbers of steps per cow per 30 minutes over the duration of outdoor access were calculated and graphed in the same manner. All calculations for descriptive data described above were completed using Microsoft Excel (Microsoft Corp., Redmond, WA).

To compare elimination rates of the first hour of outdoor access to those of the second hour of outdoor access, the initial model was again modified. The effect of access type was not included since all cows were Outdoor cows. The effect of time was removed because only Outdoor cows provided 2 hours of outdoor access were retained for this question. The variable *observation_hour* was added to denote the first hour of observation and the second hour of observation. The Latin square was unbalanced due to periods 1 through 6 not being included; as a result, some of the fixed effects were not estimable because of a lack of degrees of freedom. *period* was removed from the model because it was not estimable; the same days were not observed between the two periods. Furthermore, *period* as a main effect no longer contributed to the model due to being accounted for via nesting of *daymr. season* was removed from the model because as a main effect it no longer contributed to the model due to being accounted for via nesting of *daymr. season* was removed for via nesting of block within season. The seasons could not be compared due to a missing block in summer. Ultimately it was possible to look at the main effects of *size* and *observation_hour*, but since the original setup of the experiment involved an 8x8 Latin square there was a large portion of data missing; thus the interaction (*size*observation_hour*) could not be analyzed. The finalized model for this specific question is shown below:

 $Y_{ikmnprz} = \mu + size_i + observation_hour_z + block_{np} + day_{mr} + cow_day_{npikmr} + e_{ikmnprz}$

Where: $Y_{iknnnprz}$ is the dependent variable, the outcome mesure of the k^{th} cow in the treatment of the *i*th paddock size and *z*th observation_hour, during the *m*th period, the *n*th season, of the *p*th block, and the *r*th day; *size_i* is the fixed effect of the *i*th paddock size; *observation_hour_z* is the fixed effect of the *z*th hour (*z* = first hour or second hour of observation); *block_{np}* is the fixed effect of the *p*th block within the *n*th season; *day_{mr}* is the fixed effect of the *r*th day (*r* = Monday, Tuesday, Wednesday, Thursday, or Friday) within the *m*th period; *cow_day_{npikmr}* is the random effect of the *r*th day within the *n*th season, the *p*th block, the *i*th paddock size, the *k*th cow, and the *m*th period; and *e_{ikmnprz}* is the random error. *cow_day* was added to account for the random variation from day-to-day. The model was made into a nested model such that *day* within *season*, *block, paddock size, cow*, and *period* was a random effect and *observation_hour* was a cross-classified fixed effect. Please refer to Appendix 3 and Appendix 4 for more information about covariance structures, variance parameters, and coefficients of variance for rates of eliminations pertaining to this model.

3.4 Results

3.4.1 Objective 1 – describe eliminative behaviors at tie-stalls

Average elimination frequencies per cow ranged from 10 to 30 defecations and from 5 to 16 urinations per 24 hours (Table 3-2). Average frequencies also varied between individual cows, more so for urinations within winter cows and for defecations within summer cows (Supplemental Figure 3-1). Average durations of eliminations per cow ranged from 1 to 43 seconds for defecations and from 5 to 35 seconds for urinations (Table 3-2). Defecation durations of 1 second were instances where one "clump" of feces was passed. This type of observation only occurred twice; once during the winter within 1 minute of standing, with no previous defecations, and once during the summer in the process of standing, with a defecation 2 minutes prior.

Defecation and urination frequencies followed the same general pattern over a 24-hour period, and the seasons followed similar patterns of high and low frequencies during certain periods of the day (Figure 3-2). Eliminations were most frequent during 5:00 and from 19:00 to 20:00, peaking at 4.8% and 6.8% for defecations and 6.1% and 6.8% for urinations during the winter, and at 5.7% and 7.6% for defecations and 8.1% and 7.6% for urinations during the summer for these respective time periods. Eliminations became less frequent when lights were off, from approximately 20:30 to 5:00, and after milking, during approximately 7:00 and 18:00. Winter 7:00 saw the lowest percentages of defecations, 2.1%, and urinations, 1.3%. If eliminations were evenly distributed throughout a 24-hour period, approximately 4% of total eliminations per hour would be expected. However, the observed morning and evening peaks reached an additional 1% to 4% compared to this expectation. Notably during the summer, urinations during 5:00 doubled in their percentage frequency compared to this expectation (Figure 3-2).

Step activity per cow was the greatest for both seasons from 5:00 to 6:00, and during 17:00 and 20:00. The numbers of steps per hour at these times, respectively, were 30.5, 41.5, and 46.7 for the winter and 35.1, 48.8, and 59.0 for the summer. (Figure 3-3). Durations of lying time were the greatest when lights were off, from approximately 20:30 to 5:00. During this time, lying durations per hour peaked at 51.7 minutes for the winter and 52.9 minutes for the summer. The

seasons were not notably different in the patterns of lying durations over a 24-hour period (Figure 3-4).

Most eliminations occurred while standing, considering both standing and perching categories. Urinating while lying, defecating while perching, and defecating in the process of standing were rarely observed, from approximately 4%, 1% to 2%, and 1% to 7% out of total eliminations per cow, respectively. The percentages of urinations while perching were from approximately 1% to 11% out of total urinations per cow. These combinations of body position and elimination type above were only observed in 1 to 3 individual cows. Defecation was a more "passive" action for winter cows, in that a greater percentage of nothing/idle was observed during defecations compared to during urinations. The opposite was the case for summer Outdoor cows, where 54% of their total urinations were categorized as nothing/idle. Not viewable during the winter made up a larger proportion of activity observations due to placement of the overhead cameras, compared to during the summer. Considering all cows, the percentages of eating while defecating were double that of the percentages of eating while urinating. Activities grouped as other made up approximately 1% to 10% of observations during elimination. The maximum of 10% was a result of 1 summer Control cow, which defecated while resting on its knees for an extended period of time on several occasions. Winter Control cows numerically had the greatest percentage of eliminations falling into the gutter, as well as the lowest percentage of eliminations falling completely in the stall. Eliminations that occurred within 3 minutes of standing represented approximately 8% to 23% of total eliminations observed (Table 3-3).

The percentages of eliminations falling partly and completely in the stall out of total eliminations per hour showed differences between elimination types and between the seasons. During the winter, the greatest percentages of 64.8% and 75.0% of defecations and urinations, respectively, fell into the stall during 6:00. Another defecation peak of 60.4% occurred during 19:00. Urination peaked again twice, at approximately 66% from 17:00 to 18:00, and at 68.8% during 23:00. During the summer, eliminations into the stall were the greatest at 75.6% during 6:00 and 80.4% during 12:00 for defecations and urinations, respectively. Elimination percentages were at their lowest of 13.3% for defecations and 8.3% for urinations from 2:00 to 3:00 for the winter, whereas the summer never saw values below 35.9% for defecations and 32.5% for urinations during 3:00 and 23:00, respectively (Figure 3-5).

3.4.2 Objective 2 – determine whether outdoor access affects daily patterns of elimination

Neither the rates of defecations nor the rates of urinations between Control cows and Outdoor cows allocated to 1 hour of outdoor access were significantly different (P > 0.05; Table 3-4).

3.4.3 Objective 3 – determine whether duration of outdoor access affects elimination frequencies

Elimination frequencies ranged from 0 to 0.75 defecation events per cow and from 0 to 0.63 urination events per cow, per 30 minutes of observation during winter outdoor access. Elimination frequencies ranged from 0 to 2.0 defecation events per cow and from 0 to 1.0 urination events per cow, per 30 minutes of observation during summer outdoor access. The maximum defecation frequency of 2.0 during the summer was due to an observation period including 1 cow (Figure 3-6). For summer cows provided 2 hours of outdoor access, the additional time periods due to the differences in handling compared to during the winter averaged 12.2 minutes over 9 days observation (not plotted in Figure 3-6). Eliminations were observed during this additional time on 5 total days, with frequencies ranging from 0 to 2 defecations and 0 to 1 urination considering the entire group of cows each day.

Numbers of steps ranged from 32.3 to 112.8 steps per cow, per 30 minutes of observation during winter outdoor access. Numbers of steps ranged from 37.5 to 217.0 steps per cow, per 30 minutes of observation during summer outdoor access. During the summer, the outlier for the 0 to 30-minute period occurred on Monday of week 1 while the outliers for the 60 to 90 and 90 to 120-minute periods occurred on Friday of week 1 (Figure 3-7). During the additional time periods that summer cows provided 2 hours of outdoor access experienced, numbers of steps ranged from 15.0 to 141.3 steps per cow (not plotted in Figure 3-7).

For Outdoor cows provided 2 hours of outdoor access, the rates of defecations between the first and second hour of observation were not significantly different (P > 0.05; Table 3-5). Rates of urinations during the second hour of observation were significantly greater than during the first hour of observation (P = 0.008; Table 3-5).

3.4.4 Manure measurements

The manure measurements considering all winter days were as follows: Control cows, average (n = 22 defecations) = 1.98 L per event, 1.75 kg per event; Outdoor cows provided 1 hour of outdoor access, average (n = 19 defecations) = 3.24 L per event, 2.97 kg per event. The manure measurements considering all summer days were as follows: Control cows, average (n = 19 defecations) = 3.24 L per event, 2.97 kg per event.

61 defecations) = 2.20 L per event, 2.07 kg per event; Outdoor cows provided 1 hour of outdoor access, average (n = 48 defecations) = 1.90 L per event, 1.65 kg per event.

3.5 Discussion

3.5.1 Indoor observations – elimination frequencies and durations, manure quantities, and percentages of eliminations per category

The 48 hours of indoor video observation showed a range of 10 to 30 defecations and 5 to 16 urinations, averaging between 18.6 to 21.1 defecations and 9.2 to 11.4 urinations per 24 hours when considering all 4 combinations of season and access type. These values are numerically greater than what has previously been recorded in stanchion barn tied cows in the U.S., which averaged 15.8 defecations and 7.9 urinations (Fuller study from 1928, data published in Aland et al., 2002), and in Sweden, showing averages of 16.1 defecations and 8.95 urinations (Aland et al., 2002). The largest discrepancy in average elimination frequencies was observed in a Romanian study on stanchion barn tied cows, at 8.60 defecations and 8.40 urinations during the winter and 8.50 defecations and 7.35 urinations during the summer (Acatincăi et al., 2011). However, the present study was similar in that there were numerically fewer urinations during the summer compared to the winter. Free-stall housed cows showed averages of 9.8 defecations and 7.0 urinations, and 15.4 defecations and 9.3 urinations for two separate experiments. In both experiments of the tie-stall study, cows were not observed during milking (for 1 and 2 hours per day, respectively) so frequencies could have been greater (Villettaz Robichaud et al., 2011). Though it is possible that genetics and selection for high production animals led to our greater elimination frequencies, Aland et al. (2002) had previously determined that elimination frequencies of their late 1990's cows were not different from Fuller's 1928 cows, nor did breed did have a significant effect on frequencies in Fuller's study.

Cows at the tie-stall showed a range of durations of 1 to 43 seconds for defecations and 5 to 35 seconds for urinations, averaging between 11.8 to 13.7 seconds for defecations and 12.9 to 15.5 seconds for urinations during a 24-hour period when considering all 4 combinations of season and access type. In comparison, stanchion housed dry cows averaged 7.6 and 11.7 seconds for defecations and urinations, respectively. The elimination frequencies for these cows averaged 9.0 for defecations and 4.3 urinations per 24 hours (Sahara et al., 1990). Differences in feed type and intake relating to dry stage status may have influenced the differences between the cows of the present study. Considering the durations of time spent eliminating and therefore the

amounts of excreta produced, it does not necessarily seem like cows which eliminate more frequently do so for shorter durations in these cases of video and live observations. Winter cows in the present study deviate from this idea in that manure collection data for Outdoor cows showed greater volume and weight measurements per defecation event, with fewer total defecation events compared to Control cows. The conditions of the paddocks during the winter may have contributed to this result because it was difficult to separate the wet wood chips from the manure when shoveled, possibly contributing to overall weight and volume measurements.

The present study analyzed cow position out of total defecations and total urination events per cow per day, with cows averaging between 9% to 21% of defecations and 0% to 4% of urinations while lying. The proportions of eliminations while lying in the stall for free-stall housed cows were overall lower in comparison, at 4.7% and 8.2% for defecations and 1.5% and 0.1% for urinations (Villettaz Robichaud et al., 2011). This contrast might suggest that, when provided with an area specifically for lying and resting, cows move such that they end up eliminating elsewhere in the environment. The time between standing from a lying position and a cow's first elimination may offer one explanation. Our tied cows averaged between 14% to 23% of daily defecations and between 8% to 18% of daily urinations within 3 minutes of standing, and free-stall housed cows showed 12.6% of defecations and 21.4% of urinations (Villettaz Robichaud et al., 2011). In this time that has elapsed between standing and first elimination, cows appear to have moved to a different location in the free-stall barn while tied cows remain in the stall. In further support of this notion, cows in the present study showed 32% to 54% of nothing/idle behavior during elimination whereas the free-stall cows of the study by Villettaz Robichaud et al. (2011) showed only 2.1% to 8.8% of eliminations with 4 feet in the stall. The notion of "intentional" versus "coincidental" avoidance of feces has been previously studied in dairy cows (Whistance et al., 2007; Whistance et al., 2011), and a preference test for lying surfaces observed a tendency for cows to avoid urinating on clean surfaces (Schütz et al., 2019).

Control cows during the winter trial were the best at keeping their stalls free of excreta, numerically showing both the greatest percentages of defecations and urinations respectively to fall completely in the gutter, 62% and 63%, and the lowest percentages of eliminations to fall completely into the stall, 16% and 9%. This Control verses Outdoor contrast was not observed in summer cows, so season was not likely to play a factor. Additionally, cows were similar in terms of size and body condition score, where a relationship between smaller body size and fecal

contamination of the stall has been previously documented (Herlin et al., 1994). Milk production or days in milk for these 48 hours of observation provide potential explanations for differences between Control and Outdoor cows. High milk yield cows have shown less lying time compared to lower-yield cows of the same lactation stage in tie-stalls (Norring et al., 2012), while lying time for free-stall housed cows has been observed to increase as days in milk increased (Bewley et al., 2010). Both cases influence the placement and movement of the cow within her stall and, therefore, where her excreta falls.

The Latin square design of the original experiment limited the opportunity to statistically compare elimination frequencies and durations, and percentages of elimination events per category between winter Control, winter Outdoor, summer Control, and summer Outdoor cows. For the same reason, cow characteristics such as parity and activity levels and their potential relationships to eliminative behavior outcome measures were not analyzed. Thus, comparisons based on numerical values (raw data) were presented. The factor of individual cow has been reported to affect elimination frequencies per 24 hours, while these frequencies were not significantly correlated with stage of lactation nor milk production (Aland et al., 2002). Similarly, it appears that cows' elimination frequencies are correlated between days such that cows which eliminate more frequently do so daily, and variations seen between cows were not related to days in milk or feed and water intake (Villettaz Robichaud et al., 2011). Neither age (Fuller study from 1928, data published in Aland et al., 2002) nor parity (Villettaz Robichaud et al., 2011), furthermore, have been correlated to elimination frequencies. In this regard, future research for tie-stall barns should examine cow characteristics and potential relationships to eliminative behavior outcome measures other than frequencies per 24 hours, such as where excreta falls and the duration of time between standing and first elimination.

It does not appear that the provision of outdoor access on weekdays considerably affects eliminative behaviors during the weekends, following the routine of the present study. It should be noted, however, that icy paddock conditions during the winter prevented Outdoor cows from experiencing their outdoor periods during week 6 of the original experiment while summer cows proceeded on a normal schedule. Therefore, future studies are needed to confirm whether providing outdoor access periods for tied cows results in any sort of "carryover effects" on eliminative behaviors during days which cows remain entirely indoors.

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3.5.2 Indoor observations – hourly distributions of elimination frequencies, cow activity, and eliminations falling into stall, and relationships to human activity

We found that elimination frequencies when considering all cows appeared to increase during times when cows and humans are most active. The peaks in elimination frequencies for both seasons were during 5:00 and from 19:00 to 20:00. Aland et al. (2002) reported patterns of peaks during times of milking and feeding, though the barn involved a locked manger in which there was no access to feed from 9:30 to 12:30 nor overnight from 17:00 to 5:00. The differences between active periods and other hours of the day were likely due to these scheduled resting periods of this specific stanchion barn. In a stanchion barn without such a schedule, cows observed by Acatincai et al. (2011) also showed more eliminations during milking and feeding times. These differences between active and resting periods were more apparent during the summer, and were not as drastically different overall compared to the above study. The dry cows from Sahara et al. (1990) showed a peak in defecation frequencies only in the hour after the morning feeding and peaks in urination frequencies after both morning and afternoon feedings, also in a stanchion barn. Villettaz Robichaud et al. (2011) found diurnal rhythms of standing and time spent at the feeder for free-stall cows, but determined that they were not closely associated with elimination frequencies. Such differences between these housing systems may be due to tiestall cows being in front of the manger at all times, while free-stall cows might be at a different location of the barn during feed delivery.

Peaks of elimination frequencies in the present study coincided with when barn lights were turned on and cows were prompted to stand for milking, and when barn staff would have been preparing to leave for the evening. However, the timing of barn activities varied between seasons. The final feed delivery of the day during the summer was much earlier in the afternoon compared to the winter. During both seasons, the degrees of manipulation by the barn staff were greatest from 5:00 to 6:00 and were associated with stall cleaning (in most cases involving prompting cows to stand) and replacing bedding (also involving stall cleaning and prompting cows to stand). Frequencies of stall cleaning were low throughout the remainder of the day during the winter, and during the summer, frequencies of stall cleaning peaked again during 20:00. The second bedding replacement of the day occurred around 15:30 during the winter and around 20:30 during the summer. Therefore, barn staff activities were not likely related to the 19:00 to 20:00 peak in elimination frequency observed in both seasons.

The graphs illustrating numbers of steps per cow per 24 hours support the idea that more eliminations occur during times of activity. Step and elimination frequency graphs are similar in terms of where peaks and valleys occur, coupled with the fact that cows were observed to eliminate while standing (standing and perching categories) more than 75% and up to 100% out of total defecations and urinations per cow, respectively. To the author's knowledge, studies examining the hourly patterns of step activity for tie-stall cows do not exist. Hence, feeding and grazing activity was used here as a proxy for describing step activity over a 24-hour period on the basis that cows normally stand or walk while feeding. Lactating tie-stall cows in one study have shown two peaks in percentages of time spent eating, first during milking and second 2 hours before lights were turned off. The highest peak in percentage of time spent standing idle (defined in the study as standing and not eating, not necessarily an absolute lack of movement) was during the same hour lights were turned off (Haley et al., 2000). These timings of feeding, milking, activity, and lights were similar to what was observed in the present study for step activity. As previously mentioned, free-stall cows showed evidence of diurnal patterns of percentages of daily total time spent standing and eating. The former peaked during the hours of milking while the latter peaked the hour following afternoon milking (Villettaz Robichaud et al., 2011). Gibb et al. (1998) studied grazing activity for dairy cows at pasture and revealed 5 to 6 periods of feeding, with one occurring through the hour of sunset.

The peaks in step activity from 5:00 to 6:00, during 17:00, and during 20:00 coincided with the morning milking and feeding, afternoon milking, and when barn staff would have been preparing to leave for the evening. The numbers of steps were the greatest during 20:00 for both seasons. Following the explanation of barn staff activity mentioned previously, it is possible that this observation of a maximum number of steps per hour per cow during 20:00 is related to the 19:00 to 20:00 peak in elimination frequencies.

The durations of lying time per cow per hour were essentially the opposite of step activity. The greatest durations were from 2:00 to 4:00, with two main lying periods occurring between 7:00 to 9:00 and during 18:00. Sahara et al. (1990) reported one peak of approximately 29 minutes during 12:00, and from 19:00 to 5:00 a relatively consistent block of lying ranging from approximately 36 to 43 minutes. It is likely that the cows being in dry stage, as well as receiving only 2 feed deliveries per day, were related to these differences in lying time on a 24-hour basis.

The present study illustrated the hourly percentages of eliminations falling completely and partially in the stall out of total eliminations per each hour. To the author's knowledge, this aspect of eliminative behavior has not been recorded in previous studies involving tie-stall cows. The winter peak of eliminations into the stall during 6:00 coincided with morning milking and feeding. The second peak in defecations into the stall occurred around the time of the final feed delivery. The second peak of urinations into the stall was likely associated with afternoon milking, and while there was a third peak during 23:00, this time accounted for a small fraction of the 24-hour period's total urinations. During the summer, defecations into the stall peaked following morning milking and feeding. However, urinations into the stall were at their greatest percentage midday, possibly related to feed delivery, in addition to the increase that is expected during afternoon milking.

Times showing the greatest percentages of elimination frequencies did not necessarily equate to times where the greatest percentages of eliminations contaminate the stall. Notably, there is not a peak in the proportion of eliminations into the stall from 19:00 to 20:00 when high proportions of elimination frequencies had occurred. The high step activity of the cows during this time also did not appear to relate to a risk of excreta falling into the stall. It is expected that eliminations falling into the stall would always be associated with feed delivery due to the cows' forward positioning in the stall while eating. However, this did not appear to be the case with our cows. Ultimately the times towards the end of milking periods should continue to be areas of focus for cleaning stalls, such that more than 3 minutes after standing have passed and before cows lie down to rest after being milked.

3.5.3 Outdoor access – elimination and step activity frequencies and distributions

Elimination rates during outdoor access were not statistically different from rates observed concurrently indoors, at the tie-stall. Main effects of block, day, and season also did not have a significant effect on elimination rates. Outdoor access for the observations involved in this question occurred during 10:00, a time which did not coincide with peaks in elimination frequencies, step activity, or lying durations. Therefore, in addition to not contributing to greater amounts of excreta deposition outdoors compared to indoors, the schedule of the experiment did not appear to interfere with cows' normal daily routines (i.e., what was observed during the 48 hours of video when both Control and Outdoor cows were indoors at tie-stalls). In preparation for outdoor access each day, all experimental cows at tie-stalls were prompted to stand and more than 3 minutes had passed before bringing cows outside. It is possible that this time between standing and leaving the barn provided an opportunity for Outdoor cows to defecate and urinate before actually entering the paddocks. Such an idea has been suggested by White et al. (2001), where they described allowing their rotationally grazed cows time to eliminate after standing at pasture before being brought in for milking, thus reducing manure in the lanes to the barn. Outdoor cows in the present study were brought outside 1 pair at a time such that some eliminations occurred before all pairs were inside their paddocks (i.e., before the beginning of the set 1 hour of observation). Because all experimental cows were prompted to stand before bringing cows outside, a similar situation occurred for Control cows who remained at the tie-stall. Therefore, observation conditions were equal between access types and any eliminations prior to the start of observation influenced cows' 1-hour observations in the same manner.

The boxplots of frequencies of eliminations per cow during outdoor access illustrated how events were spread throughout 30-minute periods of time over all observation days. The spreads of frequencies for defecations were centered towards greater values during the 30 to 60 and 60 to 90-minute periods for both seasons; particularly so for the 60 to 90-minute period during the summer. The spreads of frequencies for urinations were more even throughout the 30minute periods during the winter, while during the summer, they were centered towards greater values during the second hour of observation. Anecdotally it appeared as if cows started eliminating when barn staff were preparing to bring inside the cows provided 1 hour of outdoor access. This would have consistently resulted in greater frequencies of elimination during the 60 to 90 minute time period, as it included the times that cows provided 1 hour of outdoor access were brought inside (approximately 11:00 to 11:15 each day). However, the only evidence of this phenomenon was in the summer frequencies for defecations.

The boxplots of numbers of steps per cow during outdoor access did not appear to show clear patterns of activity throughout 30-minute periods of time over all observation days. The spreads of numbers of steps also did not reflect the spreads of elimination frequencies as they did for the 48 hours of indoor video observations. Overall, cows during the summer took more steps compared to during the winter, with a peak during the 90 to 120-minute period. The seasonal

variation in step activity was likely due to summer cows being able to graze while winter cows were on wood chips.

Cows which were provided 2 consecutive hours of outdoor access did not show differences in defecation rates between the first and second hours, while the second hour of observation showed significantly more urinations than the first hour. In both analyses, main effects of block, day, and paddock size did not have a significant effect on elimination rates. The results for urination rates were consistent with the fact that cows overall urinated less frequently than they defecated in the present study, and as observed in previous studies (e.g., Aland et al., 2002; Villettaz Robichaud et al., 2011). Additionally, the average intervals between eliminations for tied dry cows in a stanchion barn were approximately 2.7 hours for defecations and 5.6 hours for urinations. Considering that the elimination frequencies per 24 hours were approximately half (Sahara et al., 1990) that of the present study, these values reflect the rates observed for the outdoor observations of our cows.

Though comparing the elimination rates between cows allocated 1 and 2 hours of outdoor access was not an objective of the present study, it should be noted that lactating cows provided time-limited pasture access did not differ in elimination rates between 4, 6.5, and 9-hour time treatments (averaged 0.37 defecations and 0.26 urinations per hour). Cows in each time group had adjusted their activity levels to maximize grazing time, which still did not affect elimination rates (Oudshoorn et al., 2008). Numerically, the difference between rates of defecations and rates of urinations are not as large as what was observed for our Outdoor cows.

3.6 Conclusion

In conclusion, observations were conducted for the eliminative behaviors of tie-stall housed dairy cows while at the tie-stall, and during periods of outdoor access for experiments held during the winter and summer seasons. Elimination frequencies were high when barn staff, and as a result, the cows, were the most active: during morning milking and feeding. An evening peak of elimination frequencies was also observed outside of barn staff activities and was likely related to the observed peak in numbers of steps per cow. Considering the durations of eliminations and therefore the amounts of excreta produced, it does not necessarily seem like cows which eliminate more frequently do so for shorter durations. Neither the times with the greatest proportions of elimination frequencies nor times of high step activity appeared to be related to hourly percentages of eliminations that fell into the stall. Elimination rates during 1

hour of outdoor access were not statistically different from rates observed concurrently indoors, at the tie-stall. For cows allocated to 2 consecutive hours of outdoor access, defecation rates during the first hour outdoors were not statistically different from rates during the second hour outdoors. However, urination rates during the second hour outdoors were approximately double the rates during the first hour outdoors. Frequencies of defecations and urinations per cow were greater during the middle and the end of outdoor periods, respectively, while frequencies of steps per cow did not show clear patterns per 30 minutes other than being greater overall during the summer. Such information will provide insights to designing and managing successful housing systems for dairy cows which allow for partial exercise access via outdoor paddocks.

Observation type	Category	Description						
Position	In process	Begins defecating in lying position then stands while defecating, or begins defecating at some point during standing						
	Lying	Body in contact with the stall floor						
	Perching	Forelegs in stall, hind legs in gutter or beyond						
	Standing	All four legs in stall						
Activity ¹	Eating	Actively grabbing and chewing feed with head down, or any chewing movement after gathering a mouthful of feed						
	Not viewable	Head/shoulders blocked by own body, neighboring cow, or out of camera view						
	Nothing/idle	No activity, including sleeping positions of the neck curled/tucked to side or the body is sprawled flat out on side with neck/head lying in manger						
	Other	Any other activity not described here, including allo-/self- grooming, being milked, drinking, walking backwards/forwards						
	Ruminating	Rhythmic/repeated chewing motions not associated with head down in feed						
Where excreta falls	Stall	All excreta falls completely in the stall						
	Partly	Excreta falls partially in the stall and partially in the gutter or beyond						
1	Gutter	Excreta falls completely in the gutter or beyond, nothing in the stall						

Table 3-1. Ethogram of cow position, activity, and where excreta falls during each elimination event for 48 h of indoor video observation.

¹ Adapted from Zambelis et al. (2019).

		Winter								Summer								
		Control			Outdoor			Control				Outdoor						
Parameter	Elimination	Ν	Min	AVG. ±	Max	Ν	Min	AVG. ±	Max	Ν	Min	AVG. ±	Max	Ν	Min	AVG. ±	Max	
	type			3D				3D				3D				3D		
Frequencies	Defecations	7	10	19.4 ± 2.73	24	8	16	19.1 ± 1.58	23	8	16	21.1 ± 4.12	30	7	16	18.6 ± 1.18	22	
	Urinations	7	5	11.4 ± 2.83	15	8	5	11.0 ± 3.33	16	8	6	9.2 ± 1.49	12	7	7	9.4 ± 0.98	12	
Elimination durations, s	Defecations	7	1	12.5 ± 2.14	26	8	5	12.2 ± 2.52	43	8	2	13.7 ± 2.74	32	7	1	11.8 ± 2.46	29	
	Urinations	7	5	12.9 ± 2.99	35	8	5	13.6 ± 2.40	34	8	5	15.5 ± 3.37	30	7	6	14.1 ± 1.80	29	

Table 3-2. Average elimination frequencies and durations per cow per 24 h of observation (rawdata). These 48 h of indoor observation were conducted via video recordings.
Table 3-3. Percentages of elimination events per category out of total eliminations per cow, averaged over 2 d (raw data). These 48 h of indoor observation were conducted via video recordings. Due to rounding of numbers, percentages may not add up to 100.

			Winter		Summer		
			Control	Outdoor	Control	Outdoor	
	~ 1		$(n^2 = 7)$	$(n^2 = 8)$	$(n^2 = 8)$	$(n^2 = 7)$	
Observation Type	Category	Elimination	AVG. \pm SD	AVG. \pm SD	AVG. \pm SD	AVG. \pm SD	
Position	Standing	Defecation	76 ± 19.4	78 ± 14.9	82 ± 15.1	83 ± 15.2	
% of		Urination	89 ± 16.2	99 ± 2.9	92 ± 15.8	91 ± 24.3	
eliminations	Lying	Defecation	21 ± 17.8	19 ± 13.3	16 ± 15.3	9 ± 10.9	
		Urination	0 ± 0.0	0 ± 0.0	4 ± 10.6	0 ± 0.0	
	Perching	Defecation	1 ± 2.0	2 ± 4.5	0 ± 0.0	0 ± 0.0	
		Urination	11 ± 16.2	1 ± 2.9	5 ± 13.3	9 ± 24.3	
	In process ³	Defecation	1 ± 3.0	1 ± 1.9	2 ± 2.6	7 ± 16.0	
		Urination	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	
Activity,	Nothing/	Defecation	39 ± 13.8	41 ± 21.0	33 ± 9.9	41 ± 14.0	
% of	idle ⁴	Urination	34 ± 32.0	32 ± 30.5	33 ± 28.9	54 ± 22.8	
eliminations	Not viewable	Defecation	34 ± 15.3	41 ± 26.4	32 ± 9.6	27 ± 8.3	
		Urination	52 ± 36.4	56 ± 37.5	48 ± 31.3	27 ± 24.5	
	Eating	Defecation	13 ± 10.8	7 ± 5.5	14 ± 12.2	19 ± 12.6	
		Urination	7 ± 6.8	4 ± 6.3	5 ± 4.7	7 ± 11.0	
	Ruminating	Defecation	10 ± 7.6	10 ± 7.1	11 ± 4.3	8 ± 9.4	
		Urination	6 ± 6.4	7 ± 6.4	11 ± 11.5	8 ± 9.1	
	Other ⁵	Defecation	5 ± 3.9	1 ± 1.2	10 ± 10.0	5 ± 4.8	
		Urination	1 ± 1.3	1 ± 2.2	4 ± 8.8	4 ± 6.4	
Where excreta	Gutter	Defecation	63 ± 16.6	54 ± 25.0	43 ± 26.8	48 ± 33.4	
falls,		Urination	62 ± 28.0	50 ± 31.1	39 ± 33.0	40 ± 38.0	
% Of eliminations	Partly	Defecation	21 ± 6.7	21 ± 9.6	24 ± 11.9	19 ± 6.4	
ciminations		Urination	29 ± 18.8	36 ± 20.6	33 ± 28.5	24 ± 22.8	
	Stall	Defecation	16 ± 15.1	25 ± 22.9	33 ± 24.1	33 ± 30.7	
		Urination	9 ± 20.0	14 ± 21.1	28 ± 32.6	36 ± 40.5	
Standing to first	Within 3	Defecation	14 ± 8.3	14 ± 9.3	15 ± 9.0	23 ± 16.7	
elimination ⁶ , % of	minutes	Urination	18 ± 13.4	16 ± 17.8	8 ± 7.4	13 ± 10.1	
eliminations							

¹ Categories are presented independently; e.g., all instances of ruminating no matter the position of the cow

 2 n = the number of cows under observation.

³ Begins defecating in lying position then stands while defecating, or begins defecating at some point during standing.

⁴ Sleep position was grouped into nothing/idle due to occurring at a low frequency with few cows observed, and similar representation of the cow's body during both behaviors. AVG.% \pm SD% for these categories (n = 30 cows) were sleep position: defecation = 0.9 \pm 2.25, urination = 0.0 \pm 0.00; nothing/idle: defecation = 37.6 \pm 14.91, urination = 37.7 \pm 28.74.

⁵ Allo-grooming, self-grooming, walking backwards, walking forwards, drinking, and being milked were grouped into other due to occurring at a low frequency with few cows observed. AVG.% \pm SD% for these categories (n = 30 cows) were allo-grooming: defecation = 0.6 ± 1.38 , urination = 0.3 ± 1.10 ; self-grooming: defecation = 1.0 ± 3.13 ,

urination = 0.0 ± 0.00 ; walking backwards: defecation = 0.7 ± 1.79 , urination = 0.4 ± 2.28 ; walking forwards: defecation = 0.8 ± 1.67 , urination = 0.0 ± 0.00 ; drinking: defecation = 0.1 ± 0.35 , urination = 0.0 ± 0.00 ; being milked: defecation = 0.1 ± 0.41 , urination = 0.2 ± 0.91 ; other: defecation = 1.8 ± 4.99 , urination = 1.3 ± 4.74 . ⁶ An elimination that occurs within 3 minutes of standing from the lying position.

Table 3-4. Comparison of rates per h of eliminations during 1 h of observation (LSmeans \pm SE) between cows not provided outdoor access (Control; n = 7-8 cows) and cows provided 1 h of outdoor access (Outdoor 1 h; n = 2-4 cows). These days of outdoor observation (winter: 3 d; summer: 4 d) were conducted live.

Outcome measures	Control cows	Outdoor 1 h cows	Ddf	F-value	P-value
Defecations, rate	1.09 ± 0.140	0.91 ± 0.190	9.26-13.7	0.64	0.440
Urinations. rate	0.60 ± 0.240	0.60 ± 0.173	3.43-4.37	0.00	0.984

Table 3-5. Comparison of rates of elimination (LSmeans \pm SE) between the first and second h of observation for cows provided 2 consecutive h of outdoor access (Outdoor 2 h; $n = 1-4 cows)^1$. These days of outdoor observation (winter: 8 d; summer: 9 d) were conducted live.

Outcome measures	First h outdoors ²	Second h outdoors ²	Df	Difference ³	F-value	P-value
Defecations, rate	0.71 ± 0.127	0.74 ± 0.127	74		0.04	0.837
Urinations, rate	$0.28\pm0.091^{\text{b}}$	$0.55\pm0.091^{\text{a}}$	74	$\textbf{-0.27} \pm 0.100$	7.56	0.008

¹ Only cows which were provided 2 h outdoor access were included in this analysis. ² Term LSmeans within a row with different superscripts (a, b) differ (P < 0.05).

³ LSmeans difference \pm SE.



Figure 3-1. Schematic of elimination observations during experimental procedure of outdoor observations.

¹ The only difference between winter and summer seasons is the approximately 15 additional min due to differences in handling of the cows.



Figure 3-2. Percentages of hourly elimination frequencies out of total eliminations for all cows, averaged over 2 d for (a) the winter (n = 15 cows) and (b) the summer (n = 15 cows) seasons. These 48 h of indoor observation were conducted via video recordings. Standard error bars are shown, and barn activities of milking, feeding, and lights are illustrated.



Figure 3-3. Numbers of steps per cow, averaged over 2 d for (a) the winter (n = 15 cows) and (b) the summer (n = 14 cows; data missing for 1 cow) seasons. Data was collected via IceTag pedometers during 48 h of indoor observation. Standard error bars are shown, and barn activities of milking, feeding, and lights are illustrated.



Figure 3-4. Duration (min) of lying time per cow, averaged over 2 d for (a) the winter (n = 15 cows) and (b) the summer (n = 14 cows; data missing from 1 cow) seasons. Data was collected via IceTag pedometers during 48 h of indoor observation. Standard error bars are shown, and barn activities of milking, feeding, and lights are illustrated.



Figure 3-5. Percentages of eliminations falling completely and partially in the stall out of total eliminations for all cows, averaged over 2 d for (a) the winter (n = 15 cows) and (b) the summer (n = 15 cows). These 48 h of indoor observation were conducted via video recordings. Standard error bars are shown, and barn activities of milking, feeding, and lights are illustrated.



Figure 3-6. Frequencies of eliminations per cow per half-hour during outdoor access for (a) the winter and (b) the summer seasons (raw data). Each box per half-hour represents the spread of data between all days of the 2 weeks of outdoor access. These days of outdoor observation (winter: 8 d; summer: 9 d) were conducted live. The number of cows observed (winter: n = 7-8 cows; summer: n = 1-7 cows) varied between days due to allocated outdoor access times (1 or 2 h) and conditions which did not allow cows to go outdoors (e.g., signs of cows in heat).



Figure 3-7. Numbers of steps per cow per half-hour during outdoor access for (a) the winter and (b) the summer seasons (raw data). Each box per half-hour represents the spread of data between all days of the 2 weeks of outdoor access. These days of outdoor observation (winter: 8 d; summer: 9 d) were conducted live. The number of cows observed (winter: n = 7-8 cows; summer: n = 1-7 cows) varied between days due to allocated outdoor access times (1 or 2 h) and conditions which did not allow cows to go outdoors (e.g., signs of cows in heat).

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3.8 Supplemental material

Supplemental Figure 3-1. Elimination frequencies per cow per 24 h, averaged over 2 d for (a) the winter and (b) the summer seasons. These 48 h of indoor observation were conducted via video recordings. Some cows remined indoors at the tie-stall for the duration of the experimental period (Control), while others were allocated to outdoor access periods during weekdays of the experimental period (Outdoor). Standard error bars are shown.

CHAPTER 4 – GENERAL DISCUSSION

Understanding the eliminative behaviors of dairy cattle is essential for maintaining the welfare of the animals and for providing producers knowledge such that outdoor paddocks, yards, or indoor exercise areas can be successfully implemented. The aim of the review in Chapter 2 was to examine the literature surrounding this aspect of behavior for dairy cattle specifically. The first point to acknowledge is that there does not exist a common definition for eliminative behaviors, which makes both providing titles for studies and searching for literature on this topic difficult (e.g., use of "elimination" verses "eliminative" verses "excretion"). Secondly, there is a general lack of information and a need for updated information regarding the typical eliminative behaviors of dairy cows housed in tie-stall systems in a North American context. It is possible that differences in region or year could mean differences in cows (e.g., breed or genetic selection over time) or what resources are available (e.g., restriction to tie-stall housing due to harsh winters), which may also affect eliminative behaviors. Though many internal and external factors can affect the eliminative behaviors of dairy cattle, this review also highlighted that there is a gap in knowledge regarding the points of exercise and movement opportunity. It is interesting that this is the case even though eliminative behaviors appear to be the most different between types of housing systems. The type of housing system in turn determines the degrees to which cows are manipulated by barn staff and the activity levels of cows; both aspects fundamental to the concept of introducing of increased exercise and movement opportunity.

The study presented in Chapter 3 involved tie-stall housed lactating Holstein cows that were part of a larger experiment examining the effects of outdoor exercise access on behavior and activity. The schedule of the original experiment provided an opportunity to both fully detail the eliminative behaviors for cows at tie-stalls on an hourly basis and begin to describe elimination frequencies when cows were provided access to outdoor paddocks. The similar numerical values for elimination frequencies, durations, position and activity during, and whether the event occurred within 3 minutes of standing between season and cow type suggest that the weekday schedule of outdoor access does not affect eliminative behaviors when cows are kept inside at tie-stall during the weekends (no outdoor access). Hourly distributions of elimination frequencies were determined to relate most closely to cow steps, with peaks and valleys at similar times of the day. It was also found that the times with the greatest proportions

of eliminations which fell into the stall did not necessarily equate to times with the greatest proportions of elimination frequencies, nor the times when cows took the most steps. To the author's knowledge, the concurrent rates of eliminations between cows indoors and cows outdoors have not been compared in previous studies. This study found that for a duration of 1 hour, elimination rates were not significantly different between cows indoors at the tie-stall and cows provided outdoor access. In comparing the first and second hours outdoors, defecation rates were not significantly different while urination rates were around double during the second hour. Defecations and urinations per cow occurred more frequently during the middle and end of outdoor periods, respectively. The numbers of steps per cow during outdoor access did not appear to be related to eliminations in the way they were while all cows were indoors at tie-stalls, but cows were overall more active during the summer compared to during the winter.

The effects of exercise and movement opportunity on the eliminative behaviors of dairy cattle are particularly relevant as the dairy industry moves away from year-long or continuous tie-stall housing systems. In any case, practical aspects of management and labor should be considered; for instance, exercise areas should be designed to account for removal of excreta. Future studies on eliminative behaviors (especially behaviors other than simple frequencies) should highlight two facets: a focus on cows provided exercise/movement opportunity, to determine whether partial access affects the remainder of their time indoors and confirm that consecutive days of partial access do not affect days which they are not provided access; and a focus on the concurrent comparison between cows which remain inside and cows which are provided longer durations of access.

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(CV) for eminiatio	on rates during	I II ODSErvation of	Control and C	Juluoor cows.	
Outcome measures	$\overline{\mathbf{X}}$	S.E.M.	σ	CV	
Defecation	1.00	0.06	0.30	0.30	

0.60

APPENDIX 1. Table showing mean (\bar{x}) , S.E.M., variance (σ) , and coefficient of variation $(CV)^1$ for elimination rates during 1 h observation of Control and Outdoor cows.

0.05

0.17

0.28

 $\frac{\text{Urination}}{^{1}\text{CV} = \sigma \ / \ \overline{x}}$

APPENDIX 2. Table showing random effects variances (σ^2_{cow} , σ^2_{day} , σ^2_e , CS), covariance parameter estimates, phenotypic variance $(\sigma^2_p)^1$, the variable means $(\bar{x})^2$, and coefficient of variation (CV)³ for elimination rates during 1 h observation of Control and Outdoor cows.

Outcome measures	σ^2_{cow}	AR(1)	CS	σ^2_{day}	σ^2_{e}	σ^{2}_{p}	$\overline{\mathbf{X}}$	CV (%)
Defecation	-	-	0.18	-	0.34	0.52	1.00	71.87
Urination	-	-	0.16	-	0.22	0.38	0.60	103.22

 $\label{eq:constraint} \begin{array}{l} ^{1} \sigma_{p}^{2} = \sigma_{cow}^{2} + \sigma_{e}^{2} \\ ^{2} \overline{x} = \text{the average between the two treatment LSmeans} \\ ^{3} CV = (\text{sqrt} (\sigma_{p}^{2}) \, / \, \overline{x}) * 100 \end{array}$

APPENDIX 3. Table showing mean (\bar{x}) , S.E.M., variance (σ) , and coefficient of variation $(CV)^1$ for elimination rates of Outdoor cows provided 2 h outdoor access, first and second h outdoors.

Outcome measures	x	S.E.M.	σ	CV
Defecation	0.73	0.06	0.37	0.50
Urination	0.42	0.04	0.19	0.45

 1 CV = σ / \overline{x}

APPENDIX 4. Table showing random effects variances (σ^2_{cow} , σ^2_{day} , σ^2_e , CS), covariance parameter estimates, phenotypic variance $(\sigma_p^2)^1$, the variable means $(\bar{x})^2$, and coefficient of variation (CV)³ for elimination rates of Outdoor cows provided 2 h outdoor access, first and second h outdoors.

Outcome								
measures	σ^2_{cow}	AR(1)	CS	σ^2_{day}	σ^2_e	σ^{2}_{p}	$\overline{\mathbf{x}}$	CV (%)
Defecation	-	-	-	0.00	0.46	0.46	0.73	92.99
Urination	-	-	-	0.00	0.23	0.23	0.42	116.25

$$\label{eq:constraint} \begin{split} & \overline{}^{1} \, \sigma^{2}_{p} = \sigma^{2}_{cow} + \sigma^{2}_{e} \\ & \overline{}^{2} \, \overline{x} = \text{the average between the two treatment LSmeans} \\ & ^{3} \, CV = (\text{sqrt} \, (\sigma^{2}_{p}) \, / \, \overline{x}) * 100 \end{split}$$