Can cattle be trained to urinate and defecate in specific areas?

An exploration of cattle's urination and defecation habits and some aspects of learning abilities

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfilment of the Requirements
For the Degree of Doctor of Philosophy
In the Department of Large Animal Clinical Sciences
Western College of Veterinary Medicines
University of Saskatchewan
Saskatoon

By

Alison Vaughan

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed with-out my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Large Animal Clinical Sciences
Western College of Veterinary Medicine
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 5B4
Canada

OR

Dean
College of Graduate Studies and Research
University of Saskatchewan
107 Administration Place
Saskatoon, Saskatchewan, S7N 5A2
Canada

Abstract

This thesis explored the feasibility of training cattle to eliminate in specific areas of a barn and investigated cattle's ability to generalise knowledge between different locations. In Chapter 2, all incidences of urination and defecation were recorded by group-housed female Holstein calves across 144 h. There were substantial differences between individual calves in the mean daily frequency. Calves urinated and defecated most frequently during daylight hours when they are more active and the location of voiding was likely related to the amount of time areas were occupied. In Chapter 3, calves were trained to urinate in a specific location via classical or operant conditioning. Classically conditioned calves were held in a stall for a set time and given no punishment or reinforcement upon urination, whereas calves in the operant treatment were immediately rewarded for urination in the stall. Classically conditioned calves did not urinate more than controls. Calves trained using operant conditioning had a higher frequency of urinations in the stall than their controls but did not seem to generalise this association; failing to urinate more than controls when tested again, 5 months later, in a new location (Chapter 4). The use of visual cues may be an effective way of helping cattle to generalise previously learned associations to a new location or context. Two experiments were conducted (Chapter 5) to investigate whether prior exposure to colour cues improves calves' performance in a Y maze colour discrimination task. In Experiment 1, either both side and colour or colour alone predicted the location of milk reward in a Y maze. Our results suggest that calves overlook colour in the presence of more salient cues, such as location. In the second experiment, calves were first classical conditioned to associate coloured signs with presence of absence of milk (colours were randomised for controls) before testing in a Y maze discrimination task. Nine out of ten classically conditioned calves, but no control calves, achieved the learning criteria. Classical conditioning can be used to rapidly train cattle to follow colour cues and generalise these associations to new contexts or locations.

Acknowledgements

Firstly, I want to thank the wonderful Drs. Anne Marie de Passillé, Jeff Rushen and Joe Stookey. I am so lucky to have you as supervisors and even luckier to consider you friends. Joe, thank you for your infectious enthusiasm, generosity and creativity. Anne Marie and Jeff, you've given me more wisdom, love and support than I deserve. I never could have done this without you. Thanks also to my committee; Dr. Christensen, Dr. Ferrari, and my many graduate chairs; Drs. Campbell, Harding and Epp, for the benefit of your advice and expertise.

Thanks to everyone at the UBC Dairy Centre, my home from home. My love and gratitude to Brad Duncan, Barry Thompson, Bill Kramer, Eric Swift, Hendrik Hanson, Mary Ann La Fond and especially my old roomie, Ted Toenders. Nelson, thank you for your friendship and for knowing the right thing to do in (almost!) every situation. Heartfelt thanks to Gosia Zdanowicz, JP Parent and the many post docs and students who helped during my PhD.

Thanks also to my dear friends scattered across the globe. We may be far but we are always close. Special thanks to Mike for showing me my path and giving me the courage follow it and to Julia and Kristi for providing high adventure and hilarity.

Finally, I'd like to thank my family for encouraging insubordination and wild ideas from an early age. Mammy, thanks for being brave and letting me find my own way. Dad, thank you for teaching me to follow my heart and trust my intuition. Malc, you are the best big brother. I love you all.

Contents

List of Tables

Table of Figures

1. Literature Review

1.1 Introduction

Management of urine and faeces produced by cattle is a challenge for the design of modern dairy facilities. Cattle produce large volumes of urine and faeces (see below) which, if permitted to accumulate, can lead to a number of cow and human health problems. Current attempts to handle manure often rely on barn designs which restrict or inhibit expression of natural behaviour and may compromise cow welfare. Manure handling and disposal also has environmental and monetary consequences. Previous studies have explored the possibility of training cattle to urinate and defecate in locations that facilitate manure handling.

1.2 Defecation and Urination

1.2.1. Overview of cattle elimination habits

Cattle have been estimated to produce approximately 31 kg of faeces and 16 kg of urine per day (Statens Jordbruksverk 1995 cited by Aland et al., 2002). Measuring the volume of faeces and urine excreted is problematic, particularly in free stall, loose housed or pasture systems and thus the majority of studies report daily frequency (Aland et al. 2002; Oudshoorn et al. 2008; Hirata et al. 2011; Villettaz Robichaud et al. 2011). To date no study has examined the relationship between total daily volume of manure and frequency of defecation or urination. One study has correlated daily frequency of defecation with volume excreted per event and found, as one might anticipate, a negative correlation (Hirata et al. 2011). This study also found an influence of activity on the faecal output per defecation with more frequent and smaller defecations occurring during active behaviours such as grazing or walking and fewer large defecations while inactive (standing or lying). Cattle display substantial individual variation in the frequency of eliminations, defecating between 3 and 36 times and urinating between 2 and 19 times per day (Aland et al 2002; Oudshoorn et al 2008; Hirata et al. 2011). The frequency of defecations is typically higher than that of urinations (Aland et al 2002; Oudshoorn et al 2008; Hirata et al. 2011). A positive correlation between the frequency of defecation and urination was found by Villettaz Robichaud et al. (2011) but not by Aland et al (2002).

Villettaz Robichaud et al (2011) reported that the daily frequency of eliminations by individuals was relatively stable across time, although this relationship was stronger for defecations. Their results indicate that 2 days of observation is sufficient to provide a good estimate of individual cows' frequency of defecation and urination occurring across 5 days. However, this finding was not supported by Aland and colleagues' 2002 study, which reported a great deal of intra-individual variation in the frequency of defecation and urination between 24 h periods.

1.2.2. Consequences of accumulation

Accumulation of faeces and urine is an important risk factor for the three major diseases of dairy cattle; lameness, mastitis and Johne's disease (Tongeľ & Brouček 2010). Wet, slippery flooring can lead to an increased risk of slips and falls, resulting in injuries (Rushen et al. 2004). Moisture can also soften the claw of the hoof making them more vulnerable to claw lesions (Rushen et al. 2004) and increasing the potential for development of infectious skin disorders of the foot (particularly digital and inter-digital dermatitis) and heel horn erosion (Hultgren & Bergsten 2001).

Contamination of bedding can also negatively impact cow comfort. Cattle preferentially lie in dry uncontaminated stalls and are reluctant to lie in soiled or wet stalls (Fregonesi et al. 2007). Thus contamination of stalls by urine and faeces effectively increases the cow to stall ratio, leading to increased competition for the remaining stalls. Dirty stalls reduce lying time which may negatively affect health and thus, indirectly, milk production (Fregonesi et al. 2007; Bewley et al. 2010). Dirty udders also reduce hygiene and increase preparation time required at milking. Appropriate and timely manure handling, and keeping stalls and cows clean all help reduce these risks but may increase labour and bedding costs.

Faecal contamination poses a risk to human and animal health and contributes towards spreading diseases, including Johnes disease (Pell 1997). The bacterium causing this disease (*Mycobacterium paratuberculosis*) can live in faeces and standing water for extended periods while remaining infective to both humans and cattle. Contamination of water sources has also been implicated in human gastrointestinal disease outbreaks (Heinonen-tanski et al. 2006). Given that milk is a food product for human consumption, good hygiene and cleanliness is of paramount importance for both consumers and dairy farm workers.

Accumulation of faeces and urine and leaching of manure into waterways also has environmental consequences. Excess nitrogen fed in the form of feed proteins is excreted in faeces and urine. Approximately 60 to 80 percent of the nitrogen in urine is in the form of urea (Vaddella et al. 2010). When urine and faeces mix, urinary urea is rapidly converted to ammonia by urease, an enzyme produced by microorganisms in faeces. It is worth noting that separately urine and faecal matter emit minimal amounts of ammonia; it is primarily the combination of urine and faeces accumulating on floor surfaces (rather than manure storage facilities, where surface area is reduced) which results in ammonia volatilisation in dairy housing.

Current methods of managing manure are not optimal for achieving the dual goals of maintaining cleanliness whilst avoiding negative impacts on cow health and comfort. There is a need for exploring alternative methods of manure management.

1.2.3. Factors influencing urination and defecation

The factors accounting for the variation found between cows in frequency of urination and defecation are not yet fully elucidated, with studies producing conflicting results (Table 1.1.). The daily frequency of defecations and urinations quoted for Fuller's 1928 study lies within the range of more recent studies (Aland et al 2002, Whistance et al 2007), which suggests that the increase in milk yields and dramatic changes in feeding practices over the last 80 years has had minimal impact on how often cows defecate and urinate.

1.2.3.1 Age

Some work has been done examining effect of age on frequency of defecation and urination but this has only included animals exceeding 11 months of age. Aland and colleagues (2002) reported significantly higher frequency of defecations, but not urinations, by cows in comparison to heifers. However this study compared just four heifers and four cows, which ranged in parity from 1-5 lactations. Given the substantial amount of individual variation in frequency of eliminations, a larger sample size would be required to draw conclusions about age effects.

Table 1.1. – ***Variables which may influence frequency of urination and defecation.***

Publication	Age	Milk production	Feed intake	Diurnal rhythm	Activity
Fuller 1928	**X**	✓ ‡*	✓	**NA**	**NA**
Sahara et al 1990	**NA**	**NA**	**NA**	✓	**NA**
Aland et al. 2002	✓ †***	**X**	✓†	✓ ***	✓ ***
Hirata et al 2011	**NA**	**NA**	**NA**	✓ ***	✓ **
Villettaz Robichaud et al. 2011	**X**	Exp 1: ✓ ‡*** Exp 2: **X**	**X**	**X**	✓ *
Erina et al 2012	**NA**	**NA**	✓ †*	✓ †***	**NA**

†defecation only ‡urination only *p<0.05 **p<0.01 ***p<0.001

"✓" indicates the paper's findings support the influence of the variables described in the column head mentioned. "**X**" indicates the paper's findings refute an influence of these variables. "NA " is used when this variable was not examined within the publication.

The majority of evidence does not support a relationship between milk yield or body weight and the frequency of defecations and urinations (Aland et al. 2002; Villettaz Robichaud et al. 2011). The lack of difference in frequency of urinations between heifers and cows in Villettaz Robichaud et al (2011) would seem to lend support to this conclusion. In contrast, the number of days cows had be lactating for (days in milk) was correlated with frequency of urination in the majority of studies. Villettaz Robichaud and colleagues' (2011) found a positive relationship in their first but not second experiment. Cows in Experiment 1 were at varying stages of pregnancy whereas those in Experiment 2 were not pregnant. During pregnancy the uterus grows and can apply pressure on the bladder triggering the urge to urinate more frequently, which may account for some of the variation seen.

1.2.3.2. Feed

It is intuitive that feed and water intake should relate to daily volume of urine and faeces however how this relates to the frequency of eliminations is not quite as clear. Fuller (1928) claims that frequency of defecation is influenced by feed intake although he provides no evidence to support this claim. Aland and colleagues (2002) found a positive tendency between frequency of defecation and concentrate intake however this was not found by Villettaz Robichaud et al. (2011). Interestingly, Erina and colleagues (2012) found the order of feed presentation (fibrous-succulent vs succulent-fibrous) had an effect on frequency of defecation which may reflect a difference in rate of passage through the digestive system.

All previous studies of elimination behaviour were conducted on cows and breeding age heifers; to date no studies have reported frequency of defecations and urinations of calves. It would be interesting and worthwhile to examine the frequency of defecation and urination in calves and the effects of changing from milk to solid feed at the time of weaning because such information could potentially be used to improve cleaning routines.

1.2.3.3. Activity

Both Aland et al (2002) and Villettaz Robichaud et al (2011) noticed a diurnal rhythm to visits to the feeders but this was only related to urination and defecation frequency in the former study. A number of studies have found a lower frequency of urination and defecation during the night when cows were less active (Aland et al 2002; Erina et al 2012) but again this was not noted by Villettaz Robichaud and colleagues (2011), who found no apparent diurnal rhythm to urination and defecation. Only one study has investigated the influence of activity on the faecal output per defecation (Hirata et al. 2011), reporting more frequent and smaller defecations occurring during active behaviours such as grazing or walking and fewer large defecations during resting and ruminating.

Cattle are known to defecate and urinate in response to stressful situations (Friend 1991), indeed frequency of eliminations has been used as a measure of fear in behavioural studies (Forkman et al. 2007). However caution must be used when interpreting such behaviours as indicative of fear; for example vocalisations are also used as a measure of fear in cattle but are in fact performed in a number of different contexts (Watts & Stookey 2000). Similarly defecation may be a result of arousal, change in activity or time of day without necessarily indicating an emotional response. Interestingly, some studies have revealed that urination in stressful situations may not just be a result of emotional leakage but may have a communicative function, alerting other cattle to potential danger (Boissy et al. 1998; Terlouw et al. 1998). To my knowledge no study has reported a negative correlation between frequency of defecation and unambiguously positive, high arousal behaviours.

The majority of urinations and defecations occur while the cow is standing, although a small portion may also occur when cattle are lying (Villettaz Robichaud et al. 2011; Whistance et al. 2011). High yielding cows defecate significantly more while lying down than low yielding cows (Whistance 2009). This was more pronounced in cows housed in cubicle systems than those housed in straw yards and may reflect difficulties in transitioning between lying and standing for cows in stalls (Krohn & Munksgaard 1993).

1.2.3.4. Control over elimination behaviour

Cattle adopt a characteristic posture during eliminations (Fig 1.1.), with the tail raised, hind feet spread and sometimes brought forward under the body and back arched, which is particularly pronounced during urination (Aland et al. 2002). These postures may reflect an attempt to minimise bodily contact with faecal matter and urine. If this does indeed reflect a conscious attempt to avoid contamination with urine and faeces during eliminations then cattle could conceivably have a preference for defecating, and particularly urinating, on more absorbent substrates. Horses are anecdotally reported to avoid urinating on hard surfaces, preferentially selecting areas with more absorbent surfaces (Ekesbo 2011). It may be possible to examine this hypothesis by comparing posture during urination on flooring offering different levels of absorbency.

Figure 1.1. – Posture adopted during a) defecation and b) urination

Cattle are typically assumed to have little voluntary control of elimination and do not demonstrate latrine behaviour. Territorial marking has been reported in other ungulate species, albeit only in adult males (Coblentz 1976). Cattle are known to avoid grazing close to areas contaminated by faeces (Whistance 2009) and will avoid lying in dirty or wet stalls (Fregonesi et al. 2007). Housing type appears to influence cattle's ability to avoid bodily contamination by faecal matter. Cattle housed in straw yards or on pasture display both incidental and intentional avoidance of areas contaminated by urine and faeces, typically moving forward after a defecation or urination event (Whistance et al. 2007). This is not possible for cattle defecating while standing in stalls, instead these cattle are obliged to step backwards through their freshly deposited faeces as they exit. Thus, although less faecal matter ends up in stall bedding, once stalls are contaminated the cow is unable to avoid contact with faeces without avoiding the stall altogether creating greater competition for lying places (Fregonesi et al. 2007, Whistance et al. 2007). High yielding cows housed in straw yards show greater incidental avoidance of faeces (moving away from area after eliminations) than low yielding cows (Whistance 2009) although the reasons for this are not clear.

1.2.3.5. Current attempts to manage manure

Management of manure poses challenges in both pasture based and indoor housed systems. Cattle show no evidence of latrine behaviour and consequently waste collection and disposal is a major labour, cost and design consideration in modern dairy barns. However, restrictive management practices designed to separate cows from their faeces often impact other unrelated behaviours, such as transitions between standing and lying (Cook 2009) and social behaviours (Krohn & Munksgaard 1993). Additionally, concrete (the preferred material for barn flooring) offers advantages related to engineering, durability and cost but exposure to hard, abrasive and unhygienic floors increases the risk of claw lesions for cattle. Cubicle systems aim to allow cattle sufficient space in which to sleep comfortably whilst ensuring they are positioned correctly for faeces and urine to fall into the manure alley behind the stall. In reality, stalls offer a choice between two negative outcomes. If stalls are too small they may lead to reduced lying behaviour and increased risk of lameness as a result of reduced time standing in the stall. If stalls are too large cows may lie incorrectly, urinating and defecating in the stall which increases the risk of mastitis and bedding and labour costs (Cook 2009).

Typically, urine and faeces collect in the manure alley creating a wet slippery surface which increases the risk of slips and falls (Rushen et al. 2004). Manure is removed from the alley using either manual or automated scrapers or a flush system. Increased alley scraping frequency keeps the barn cleaner but is a risk factor for foot lesions and may cause cows to stumble (Stefanowska et al. 1999). Some free stall barns use slatted flooring to allow manure to drain into a pit below however this flooring hinders cattle locomotion (Telezhenko & Bergsten 2005) and was found to be the least preferred flooring in preference tests (Lowe et al. 2001)

The incidence of lameness and of hoof problems is higher when cows are kept in free stalls rather than on straw packs, likely due to the softer and more secure footing for cows (Somers et al., 2003). While bedded packs offer advantages in terms of cow comfort, management of these systems poses a challenge, with increased labour and increased risk of mastitis. Clearly there is a need for an alternative solution to the accumulation of manure in barns which can provide a clean environment without compromising cow comfort or freedom of movement.

1.3. Previous attempts to toilet train cattle

1.3.1. Punishment based techniques

The idea of controlling where cattle urinate and defecate is not a new one. For example, electric cow trainers suspended just centimetres above the cows' backs are commonly used to train cattle housed in tie stalls to take a step back before urinating or defecating, thereby avoiding soiling the stall. The fact these are common devices in tie stall dairy farms certainly suggests cattle are both conscious of when they are defecating and urinating and have some degree of control over elimination behaviour. Paradoxically, the ability of cattle to exert control over, or even demonstrate an awareness of, their eliminations has been widely dismissed (Hafez & Schein 1962; Brantas 1968). Electric cow trainers are an unsatisfactory solution to reducing contamination of cow bedding for multiple reasons. A large epidemiological study of Swedish dairy cattle found use of electric trainers was a risk factor for silent heats, clinical mastitis, ketosis and culling (Oltenacu et al. 1998). It was also reported that the negative effect of diseases on reproductive performance was greater for herds with electric cow trainers. The reasons for this are not clear but it could be that the electric trainers contribute to increased stress, which can impact on health. Further study is required to test this hypothesis.

Hultgren (1991) and Bergsten and Pettersson (1992) found electric trainers do influence excretory behaviour, resulting in cattle urinating and defecating more frequently in the manure alley than those cattle housed in stalls without electric trainers. However these differences in elimination behaviour were only found to improve cleanliness in the latter study. In fact, the efficacy of electric trainers in improving cattle cleanliness was not supported by the findings of Zurbrigg and colleagues (2005), whose study of over 300 tie stall barns reported that dirty udders and hind legs were almost 20% more prevalent in farms which had electric trainers above stalls in comparison to tie stalls without. Electric cow trainers have to be adjusted to the height of the cow and it may be that incorrect placement of electric trainers could account for the variability in efficacy. Perhaps the most damning feature of electric trainers is that they are designed for use in tie stall systems. Welfare concerns and negative public perception of restrictive housing systems has led to a shift towards group housing in a number of farmed species and it seems likely the dairy industry will have to follow suite, whether voluntarily or through the introduction of new legislation.

A small pilot study examining the feasibility of controlling where two loose housed heifers urinated and defecated was conducted by Grajczyk and Baber (personal communication). Similar to electric trainers, their protocol punished heifers with electric shock if they eliminated in the straw bedded area of their pen. A transmitter was mounted in the pen and the voltage and range was adjusted so that coverage encompassed only the straw area. A unit comprising of a receiver and electric shocker was attached to the base of the heifer's tail. The action of raising the heifer's tail completed the circuit to turn on the receiver. If this occurred within the range of the transmitter (i.e. in the straw bedded area) then the receiver picked up the transmitter signal, turning on the shocker. This approach has the advantage of consistency, ensuring every instance of elimination in the incorrect area resulted in the heifer receiving an electric shock, however it lacks specificity; heifers were also penalised for other behaviours which involve raising their tail (particularly inappropriate when engaging in comfort behaviours such as turning to scratch, stretching, etc.). A system punishing lifting the tail (whether raised as part of elimination or in the action of an unrelated behaviour) whilst within a poorly defined area of the pen would penalise slow learners even more severely. Additionally, no cue other than flooring substrate (straw bedding vs concrete) was given to allow heifers to distinguish between areas were elimination was permitted (concrete) and areas where eliminations would be punished with an electric shock (the straw bedded area). While we know cattle can distinguish between different types of flooring and display preferences for standing and lying on different flooring substrates (Lowe et al. 2001; Tucker et al. 2006; Telezhenko et al. 2007), it may be that they do not present cattle with a sufficiently salient cue to define an area. Additionally, the absorbent properties of bedded areas may further reduce contamination of the hind limbs during defecation and urination in comparison to concrete floored areas. If so, this may further explain a possible preference for defecating and urinating on bedded areas.

Unfortunately this study did not use continuous observation to record the frequency of attempted eliminations on the bedded area, merely observing heifers for an unspecified amount of time each morning. Experimental heifers were housed with non-experimental heifers so it was also not possible to count the number of new defecations in the bedded area. Nonetheless, cattle typically urinate and defecate after rising (Villettaz Robichaud et al. 2011) and it was observed that the two heifers attempted to eliminate after rising for the first three days of the trial but thereafter did not attempt urination and defecation until they had left the bedded area. However, subsequent removal of the apparatus saw an immediate return to eliminating within the bedded area.

This study was too small in scale, too short in duration (heifers were observed for just 6 days) and lacked the necessary controls to draw conclusions about the efficacy of this training method.

Nevertheless, it does present an innovative approach towards toilet training cattle with many advantages (automated, target area can be gradually reduced in size) which is worth exploring further using training methods which focus on reinforcing desirable behaviours (i.e. rewarding eliminations in a desired area) rather than punishing eliminations in undesirable locations. The use of reinforcement and punishment in training will be discussed in more detail in later sections.

1.3.2. Reinforcement based techniques

More recent attempts to control the elimination habits of cattle have used positive stimuli to reinforce the desired behaviour (Saitoh et al. 2005; Whistance et al. 2009). Saitoh and colleagues (2005) aimed to reduce the eliminations occurring on a bedded area immediately after standing by encouraging heifers to move towards a feeding station after switching between lying and standing. A transition between lying and standing behaviour was detected using a device mounted to the hind leg. These devices communicated with a feeding station to allow heifers immediate access to the feeder for a limited time after standing (60 min d 1-25, 10 min d 26-51). The number of faecal masses in the resting area were counted and the bedded area cleaned twice a day. Training resulted in a decrease in the number of faecal masses on the bedded area when compared with the 16 days preceding the beginning of the experimental phase. There was a trend towards an increase in defecation in the bedded area in the second experimental phase (d 26-51). This may be an effect of reduced motivation to approach the feeder as the effect of novelty wears off over time. Alternatively, the learned response may have been extinguished as a result of failed attempts to claim feed from the feeder within the allotted time (reduced from 60 min to 10 min in the second experimental phase). A feature of operant conditioning is that the behavioural response is extinguished over time if not reinforced but, if behaviour is reinforced again, spontaneous recovery of the response occurs. It would have been interesting to see if increasing the duration of the access to the feeder back to 60 min would have resulted in a reduced frequency of defecation in the bedded area.

An interesting feature of this approach, known as counter conditioning, is that it does not attempt to reinforce or punish the target behaviour (in this case elimination) but rather focusses on training cattle to perform a behaviour which, in most instances, is incompatible with post-lying elimination on the bedded area. Counter conditioning is an effective approach to reduce or extinguish undesirable behaviours without the use of punishment. The context in which the undesirable behaviour occurs is first identified and then the animal is trained to offer a response which is incompatible with the problem behaviour. This approach has the advantage that it is often easier to train a relative simple response to replace the undesirable behaviour than to try and build a negative association with a more complex or innately rewarding behaviour. The disadvantage of using this approach to train cattle to eliminate in specific areas is that it only targets post-lying eliminations. Although 95% of cows urinate or defecate after standing (Aland et al. 2002), this accounts for just 12% of defecations and 21.4% urinations across 24 h (Villettaz Robichaud et al. 2011). Furthermore, there was a trend towards an increase in the frequency of defecation in the bedded area as the experiment progressed.

In a more recent attempt to toilet train heifers experimenters first built an association between elimination events by the heifer and grain reward, using a secondary reinforcer (a “clicker”) upon elimination to signal the availability of the reward (Whistance 2009). Heifers were held within the target area of the pen (a concrete floored area with feeders) during this phase to prevent eliminations occurring in the bedded area being rewarded. The number of urinations and defecations performed within the 4 h training session is likely to have varied widely between individuals (Villettaz Robichaud et al. 2011) and one might expect some individuals would have received many more training opportunities than others, however the frequency of eliminations within training sessions was not reported on an individual basis (Whistance 2009). Heifers appeared to form an association between elimination and the opportunity to gain a food reward, as evidenced by the fact they looked at or moved towards the trainer immediately before, during or immediately after every elimination event.

In subsequent training sessions heifers were given access to both concrete and bedded areas of the pen but only eliminations in the target area were rewarded. All heifers eliminated more on the target area than the bedded area however this is consistent with studies of untrained cattle housed in a similar straw yard and thus is not necessarily evidence of having learned (Whistance et al. 2007). Furthermore, no baseline measurements of frequency of eliminations in different areas of the pen were taken before training commenced so it was not possible to see if this was a result of training or just a reflection of usage of the different areas of the pen. Although no evidence of training having influenced the urination and defecation distribution during testing sessions was found in this study this may not necessarily mean learning did not occur.

Alternatively, heifers may not have been sufficiently motivated to traverse the length of the whole pen from the bedded area to the trainer's location to obtain their grain reward, preferring to do this only when they were in close proximity to the trainer.

1.4. The learning abilities of cattle

Since sensory, cognitive and locomotor abilities vary widely between (and indeed, within) animal species, it is important to explore learning abilities within species appropriate contexts.

1.4.1 Associative learning - Classical conditioning

To build an association between a stimulus (a specific cue or location) and a behaviour, in this case defecation or urination, we can use either classical or operant conditioning. Classical conditioning is a form of associative learning where a conditioned stimulus (CS) is paired with an unconditioned stimulus (US). Usually the CS is biologically neutral and the US is a biologically potent stimulus which elicits an involuntary unconditioned response (UR) from the animal. After repeated pairings the behavioural response can be induced by the CS alone. For example, the action of being milked (US) is linked to the milk let down response (UR). After a few milkings, milk let down is often induced by simply entering the milking parlour or even the crowd pen (CS) (Willis & Mein 1982). The novelty of the conditioned stimulus is important in the speed with which the classically conditioned response is learnt.

When training cattle to eliminate a specific location it would be an advantage to use automated training based on classical conditioning procedures, which simply require defecation and urination to be paired with a chosen stimulus until an association is formed. However, classical conditioning may not be the optimal approach to teach cattle eliminate in a specific location. Wredle and colleagues (2004) found that classical conditioning was not an effective method to teach heifers to approach a feeder in response to an auditory cues but heifers rapidly acquired the desired response through operant conditioning.

1.4.2. Associative learning - Operant conditioning

Operant conditioning is a process of behavioural modification in which the likelihood of a specific behaviour is either increased or decreased using reinforcement or punishment, respectively (Table 1.2.). Although many studies use positive reinforcement to train cattle, when moving cattle on farm negative reinforcement or positive punishment is almost exclusively used.

Table 1.2. – Description of operant conditioning

	Punishment	Reinforcement
Positive	**Positive Punishment** Addition of noxious stimulus reduces frequency of behaviour	**Positive reinforcement** Addition of appetitive stimulus increases frequency of behaviour
Negative	**Negative punishment** Removal of appetitive stimulus reduces frequency of behaviour	**Negative reinforcement** Removal of noxious stimuli increases frequency of behaviour

Operant conditioning can be used to train cattle to approach or avoid a location. In 15 days cattle learned to avoid a feed trough in response to receiving electric shocks initiated when they entered an exclusion zone around the feed trough (Lee et al. 2007). All 10 heifers learned to avoid the feed trough when trained using operant conditioning techniques, (shock ceased when the cow stopped to approach or moved away from the feed, regardless of whether she remained in the exclusion zone). In comparison, just two out of ten heifers learned to avoid the area using the uncoupled stimulus-response training method (shock continued while cow remained in the exclusion zone, regardless of her behaviour). Applying and removing aversive stimuli in response to an animal's behaviour, rather than its location, is a more effective way to train a cow since she can quickly avoid or stop the unpleasant stimuli through modification of her behaviour.

In the majority of studies using "invisible" (electric) fencing the boundary was not defined, meaning a degree of trial and error was required by cattle to determine the dimensions of the exclusion area. There is a large body of evidence which shows the detrimental effects of unpredictable punishment. Stress has a detrimental effect not only on health and milk production (Rushen et al. 2001; Duff & Galyean 2007) but is also likely to impair performance in learning tasks (Conrad 2010). Exposure to unpredictable aversive stimuli can bring about a state known as "learned helplessness" whereby an animal subjected to random aversive stimuli ceases to actively attempt to avoid subsequent aversive stimuli (Maier & Seligman 1976). Thus such an approach may be at best stressful to the cattle and at worst counterproductive, resulting in an unresponsive animal which is no longer response to training.

The use of noxious auditory stimuli as an ethical alternative to electrical shocks to train cattle to avoid an area has also been explored (Umstatter et al. 2013). However, following repeated exposures, cattle habituate to alarming auditory stimuli (in this study the sound of dog barking/human crying) as no negative outcome is ever associated with them. In contrast, irritating sounds were claimed to become increasingly aversive with increasing exposure. Although the authors reported a slight but significant decrease in the time spent in the exclusion area while sound was playing in comparison to control periods, the cattle spent the majority of their time in the exclusion zone regardless of which sound was being emitted. Additionally there is no evidence that noxious auditory stimuli are any less stressful than an electric shock (Pajor et al. 2003). Moreover, auditory stimuli are difficult to deliver on an individual basis, essentially spilling out into the surrounding area, which could negatively impact on cows that are not in violation of a ‘rule’. Use of auditory, visual or physical cues to define the boundary of exclusion areas, followed by an electric shock only if cattle continue to encroach into the prohibited area, could help cattle to map the boundary while minimising the frequency of electric shocks. If some individuals are slower learners, the addition of a cue to predict the electric shock may help to attenuate the physiological measures of stress, even if the shocks remain unavoidable (Price 1972). However, taking the alternative approach of using rewards to positively reinforce elimination in a target area would avoid the negative welfare risks and potential learning impairments inherent in punishment based approaches altogether.

Wredle and colleagues used food as a reinforcer to reward cattle approaching a target area in response to an auditory cue (2006). In this study a small box emitting an acoustic signal was attached to a collar on each cow and cows were rewarded for visiting the target area in response an audio cue. Cattle were more likely to visit the target location following the sound cue in comparison to control periods. However the response was variable and cattle were only likely to approach if they were in close proximity to the target location when the signal was given. When designing latrine areas for cattle these must be both easy for cattle to access and offer a reward sufficiently attractive to motivate cows to visit them, particularly after transitioning from lying to standing when the need to eliminate is more urgent.

1.4.3. Generalisation of learning

Training cattle to eliminate in a predetermined area requires a degree of specificity as cattle must discriminate between areas where urination and defecation is accepted or even rewarded and other areas where urination or defecation is undesirable as it either goes unrewarded or may incur a punishment. In addition to discriminating between these areas it would be highly desirable that cattle can generalise this trained response to new locations and context.

The ability of cattle to generalise learned associations to new locations or contexts is, in many cases, desirable; it would be disadvantageous to have to retrain the same task each time the cow is moved to a new pen. In contrast, it may be advantageous that some learned associations remain specific. For example, de Passillé and colleagues (1996) found that calves which had experienced aversive handling in a novel, "treatment" pen readily learned to avoid aversive handlers but did not appear to generalise this experience to their home pen. It was also noted that some calves initially developed a generalised fear of people as a result of aversive handling, and required positive handling to overcome this. In another study (Wredle et al. 2004), cattle successfully learned to approach an automated feeder in response to an auditory cue using operant conditioning procedures. This response was still presented when retested almost 1 month later but not when moved to a different location. Clearly it is not automatic that an association learned in one location will be generalised to a new location without additional training. The ability of cattle to generalise a learned association to different contexts has not been explored, however horses do not appear to immediately utilise a previously learned visual cue to solve a novel task (McCall et al. 2003).

The degree to which an association is specific or generalised varies depending on a number of factors, such as the predictability, specificity and novelty of cues used. The consistent use of novel cues, which are specific to the trained task, increases the likelihood of generalisation to a new location or context. Additionally, training in multiple locations will help animals generalise to a novel location. From a practical perspective, conducting latrine training in multiple locations is unlikely to be feasible on farm but providing cattle with highly specific, novel cues may offer a useful, practical tool to improve ability to generalise latrine training across novel locations and contexts.

1.5. Factors influencing learning abilities

Many studies investigating learning abilities in cattle have found large variation between individuals' performance in learning tasks (Kovalcik & Kovalcik, 1986; Hagen & Broom, 2003; Whistance et al., 2009). A multitude of factors can influence performance in a learning task. The main factors will be explored in the following sections.

1.5.1. Sensory abilities of cattle

To design effective protocols for training it is important to first understand how cattle perceive and process sensory information. The dominant environmental cues controlling behaviour are species-specific according to circumstances. Species-specific hierarchies may also exist for environmental cues in cattle (as illustrated in Fig 1.2.). There are some ethological studies which describe the approach of herbivores to optimum grazing. Initially, when spatially and temporally far from food resources, herbivores can employ knowledge of space, time and memory to forage; allowing sufficient time for regrowth to occur before revisiting sites (Broom 1991). Secondly, when they are still some distance from, and unable to smell food, they can locate optimum places to graze with their eyes and ears. Wild herbivores use both visual and auditory cues to locate areas where resources are located (Howery 2013). Thirdly, when the distance to the forage is close, they can select it by smell and taste.

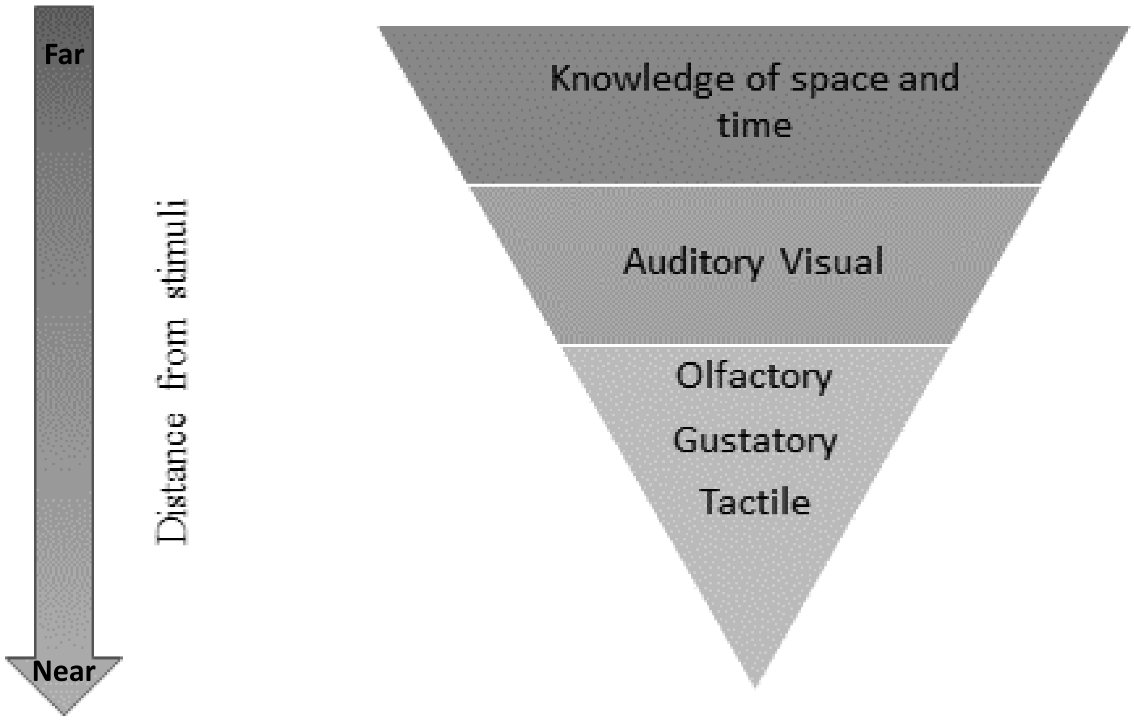


Figure 1.2. – Hierarchy of cues

Cattle rely heavily on spatial cues as these are used daily to navigate within their environment. Cattle have excellent spatial memory and can remember not only where they have foraged but also the relative quantities of food in each location (Bailey et al. 1989; Laca 1998). Cattle typically do not graze pasture evenly, instead selecting pasture according to time of day, feed quality and proximity to resources such as water troughs (Bailey 1995). Once trained to follow visual cues these can be used to direct cattle to certain areas of the pasture (Howery et al. 2013; Renken et al. 2008), however the use of visual cues for controlling the movement of cattle in indoor housing systems has not been well researched.

The eyesight of cattle is strikingly different from our own and it is important to design visual cues for cattle accordingly. Cattle have an acute sense of vision; their wide set eyes allow 330º panoramic vision, 25-50º binocular. Blind spots are located behind the cow and in the small area directly in front of their face. Cattle are adept at detecting movement but have difficulties to focus on close objects. In species with similar eye placement such as the horse, performance in discrimination tests is improved when objects are presented at ground level rather than eye level

(Hall et al. 2003). Despite this, stimulus height is rarely considered in experimental design, with many cattle studies providing visual cues at head height (Schaeffer & Sikes 1971; Uetake & Kudo 1994; Rehkämper et al. 2000; Coulon et al. 2011) or failing to specify stimulus height altogether (Entsu et al. 1992; Coulon et al. 2009). Using large visual cues is likely to make cues more obvious and easier to see. Cattle have oval shaped pupils when contracted, with the long axis nearly horizontal. This feature allows them to distinguish vertical stimulus more easily than horizontal stimuli (Rehkämper et al. 2000). This may in part account for cattle performing better at distinguishing between people wearing different colour coveralls than using other visual features (e.g. height, face) (Munksgaard et al. 1997). The colour of cues is also important. Cattle can discriminate between long wavelength colours (yellow, orange and red) but have difficulty with shorter wavelengths (blue, grey and green) (Phillips & Lomas 2001) and it has been suggested that food associations may be more easily learned with some colours than others (Uetake & Kudo 1994).

Auditory cues have been traditionally used to call cows to the milking parlour. This has been replicated in an experimental setting, using music to encourage visits to the milking unit, although success was reduced with increasing distance from the milking unit (Uetake et al. 1997; Wredle et al. 2006). Operant conditioning techniques have also been successfully used to train cattle to approach a feeding stations in response to an auditory cue (Wredle et al. 2004).

The barn and milking parlour can be noisy places and cattle may also make associations between sounds in their environment and negative experiences. In addition, the noises encountered during handling may be novel to the cattle and as a result the sounds may be more frightening (Waynert et al. 1999). Handlers commonly make a lot of noise when moving cows but shouting has been demonstrated to be as aversive to dairy cows as being hit or receiving an electric shock, although it may not perceived as such by handlers (Pajor et al. 2003). Using audio cues either as an warning of an electric shock or as an aversive stimulus itself has been explored in the context of virtual or invisible fencing (Bishop-Hurley et al. 2007; Umstatter et al. 2013).

When presented simultaneously with both visual and auditory cues cattle perform no better than with visual cues alone (Uetake & Kudo 1994). Similar results exist from the human literature. When tone and light stimuli were presented simultaneously, subjects were sometimes unaware a tone had been sounded (Colavita 1974). Interestingly, when an additional stimulus was added, in this case a haptic (tactile) cue, this attentional bias was no longer apparent. This suggests that, although vision can dominate both auditory and sensory cues, it may be limited to bi-sensory combinations (Uetake & Kudo 1994). It may be possible to influence this hierarchy through training but this has not been investigated experimentally.

Obviously sensory abilities are likely to vary between individuals and this could affect their performance in learning tasks. Currently no attempts have been made to assess the prevalence of visual and auditory impairments in dairy cattle, but studies in other domesticated species which have undergone intensive selection for production traits have revealed reduced visual acuity in comparison to their wild counterparts (Roth & Lind 2013). Interestingly, an association between deafness and pigmentation has been recorded in a number of domestic species; for example horses and canines with predominantly white faces have been reported to have a higher prevalence of deafness (Platt et al. 2006; Magdesian et al. 2009). Research is required to estimate the prevalence of compromised sensory abilities cattle and if this is associated with certain coat markings or breeds.

1.5.2. Motivation/arousal

Learning performance is not only influenced by cattle's ability to perceive stimuli but also by their level of arousal and motivation to participate in the task (Yerkes & Dodson 1908). The Yerkes-Dodson law states that there is an optimal level of arousal for performance in a learning task and too little or too much arousal can adversely affect task performance. Optimal arousal levels are considered to be task-specific, such that challenging tasks are performed more successfully at low arousal levels and simpler tasks can be performed successfully at higher arousal levels. Where arousal drops below optimal level, under-stimulation may result in slow performance or lack of interest in performing at all, both of which negatively impact speed of learning. In contrast, if arousal increases above the optimal level, performance suffers due to narrowing of focus so that only a few cues can be attended to.

There is a large variation between cattle in reactivity and this can influence arousal levels and thus performance in learning tasks. Arousal can be modified by altering the intensity of the reward or punishment, however the variation between cattle in reactivity makes it difficult to select an appropriate intensity of reinforcement or punishment accordingly. For this reason an individual approach is recommended or, where this is not possible, rewards should be used in place of punishments particularly for the learning of complex tasks as the consequences of inappropriate intensity are more detrimental with overly forceful punishment.

The level of motivation is also influenced by a number of factors such as age of animal, time since last feeding (if using food reward), and even time of day. It is important to consider all of these factors when designing a training protocol. Remarkably little work has been done to address what cattle find rewarding. It has been established that offering food rewards improves cattle's motivation to visit automated milking units more than the milking process itself (Prescott et al. 1998). Unsurprisingly cattle preferentially select large food rewards over small food rewards (Bailey et al. 1989). The quality of a reward also influences cattle's motivation to gain access it. For example, calves were found to work harder for full body contact with another calf rather than head contact alone (Holm et al. 2002). Motivation to access a reward may also be influenced by internal states, for example hunger increases cows' willingness to work for a feed reward (Cooper et al. 2010).

1.5.3. Age

Training in many domesticated species is typically targeted at young animals, as associations are most readily formed during this "sensitive period" (Bateson 1979). This is in contrast to cattle who experience relatively little interaction with humans until they are of breeding age.

Few studies have investigated the effect of age of the learning ability of cattle. Kovalcik and Kovalcik (1986) reported 15 month old heifers were faster than multiparous cows at learning which of two feeders contained food but failed to perform the task when presented with the two feeders 6 weeks later. This was interpreted as heifers failing to remember their initial training but could have been a result of greater exploratory tendencies of heifers. It has also been suggested that the concentration of heifers may be affected by the timing of the oestrus cycle and this could affect the results of training experiments (Entsu et al. 1992). Evidence of oestrus cycling was noted in the heifers during this experiment and they were found to be more easily distracted during the discrimination task on these days.

Jago and Kerrisk (2011) used pre-calving training to improve the rate of voluntary visits to an automated milking system by dairy cattle kept on pasture. Although training may have influenced behaviour prior to milking, heifers learned to use the on-farm gating system and achieved their first voluntary milking quicker than cows irrespective of the level of training. The greater learning speed displayed by heifers may also relate to reduced cognitive plasticity in older animals.

Sensitive periods for the introduction of novel stimuli are identified in a number of species and it may be that cattle become increasingly neophobic with increasing age. An increase in startle response and reduced performance in learning tasks was found in calves when they were tested at 6 weeks in comparison to 2 weeks of age (Lauber et al. 2006). This may be indicative of a curve of neophobia, found in other species.

1.5.4. Previous experience

The environment an animal is reared in also has a significant impact on their cognitive ability. Calves permitted early social contact exhibit less fear in response to novelty and reduced latency to learn to use automated feeders in comparison to calves reared in isolation (De Paula Vieira et al. 2012; Fujiwara et al. 2014). This has been attributed to reduced cognitive function in individually housed calves, a finding supported by the results of Daros et al. (2013) and Gaillard et al. (2014) who reported a greater number of errors by individually-housed vs pair-housed calves in visual discrimination reversal tasks. In keeping with the findings of studies in rodents (Schrijver et al. 2004), learning speeds of the initial discrimination task were similar between the two rearing treatments with the difference in cognitive ability only becoming apparent in the reversal stage. Deficits in reversal learning are generally considered to indicate a lack of behavioural flexibility.

The common practice of individual rearing calves may account for the findings of Grandin and colleagues (1994), who found that heifers were reluctant to change their choice of sides in a Y maze when the handling treatments offered in each side were switched. However, it is worth noting that the reversal phase of this experiment only consisted of 6 sessions and socially housed calves in Daros and colleagues (2013) required on average 10 sessions to achieve the success criterion in the reversal phase. Inflexible side preferences can also be seen in adult cattle resulting in increased time to enter the milking parlour and increased agitation during milking on the non-favoured side (Hopster et al. 1998).

Rearing animals in physically and socially complex environment presents them with many learning challenges. Animals do not only learn isolated tasks but are able to apply these learning experiences to solve similar classes of problems, known as learning sets (Harlow, 1949). Thus exposing cattle to cognitive challenges (or more complex environments) at an early age could help to maintain learning plasticity.

It would be advantageous for future research to identify an optimal age for training and examine the effects of a more complex environment which may help older animals to cope with novelty and learning challenges.

1.6. Learning Errors

Studies involving training cattle to perform a task generally report binary outcomes. The animal is considered to have learned/not learned the required task based on whether the correct response is offered. However, much like a dog intentionally ignoring its owners' recall commands in the park, there are many alternative explanations beyond failure to understand the task why the desired response may not performed. Examining the types of errors made by animals can offer us an insight into why animals are making these mistakes. However few studies explicitly report the errors made in learning tasks.

Interestingly, in the reversal experiment of Daros and colleagues (2013), which involved a go no-go colour discrimination task, the mistakes made by individually reared calves were not equally spread between the rewarded and unrewarded coloured screens. These calves readily learned to approach the previously punished colour following reversal but persisted in approaching the previously rewarded teat, despite now receiving punishment. This behaviour may be indicative of a lack of impulse control rather than a failure to learn.

So how can we discern if cattle understand that they are making an incorrect choice? In preverbal infants the violation-of-expectation method is used widely to investigate understanding (Simon et al. 1995; Wang et al. 2004; Hespos & Baillargeon 2008). In the standard version of this paradigm the subject sees an expected event, which is consistent with his/her expectation, and an unexpected event, which violates this expectation. Infants usually look longer at the unexpected than at the expected event; this indicates that they detect the violation in the unexpected event, and respond to this violation with increased attention. This technique has also been used to investigate comprehension in a range of other species such as dogs and primates (Santos & Hauser 1999; Müller et al. 2011). In cattle similar techniques, examining the behaviour during correct and incorrect choices, could reveal if errors are truly a result of failure to differentiate between the choices. Understanding why cattle make errors would not only allow us to examine their cognitive abilities but would also allow targeted improvements to training schedules.

1.7. Welfare Implications

Modern intensive production systems are often tightly controlled, allowing the cow little immediate control of her environment. There is a substantial body of work which would support the hypothesis that this lack of control may represent a source of constant stress, with both behavioural and physiological effects (Maier & Seligman 1976; Bassett & Buchanan-smith 2007). Experiences with an uncontrollable environment have been demonstrated to impact the ability to acquire a learned response or result in learned helplessness, whereby the individual ceases to interact with their environment. Automation of many husbandry tasks offers the opportunity for cattle to be active participants in their care, for example in automated milking systems cattle voluntarily visit the milking unit and are not restricted by an artificial time budget.

The welfare benefits of training go beyond simply reducing negative welfare; incorporating cognitive challenges to the dairy environment may act as a form of enrichment. Hagen and Broom (2004) found that heifers demonstrated a positive emotional response to solving a simple operant task. There is also some evidence to suggest that some individuals may seek cognitive challenges and find cognitive tasks inherently rewarding (Langbein et al. 2009). Prior learning experience may also increase flexibility of adapting to new environments and learning of subsequent tasks and is likely to result in reduced stress for both cattle and handlers.

The use of training using positive reinforcement techniques has transformed the handling and treatment of both laboratory and zoo animals; tasks previously requiring restraint or sedation are achieved without stress or risks of injury to animal or handler and controlling the movement of animals is now achieved with ease (Grandin et al. 1995; Bloomsmith et al. 2003). The rise in automation in dairy farming offers the chance to achieve similar goals in large scale production and this is an opportunity we should embrace. Increasing our knowledge of how cattle learn and what they are capable of learning is vital for fully utilizing this opportunity. Using training to address the problem of accumulation of urine and faeces in dairy barns without resorting to restrictive housing designs could lead the way to a cleaner environment and improved cow comfort and health.

References

Aland, A., Lidfors, L., Ekesbo, I., 2002. Diurnal distribution of dairy cow defecation and urination. *Applied Animal Behaviour Science*, 78, pp.43–54.

Bailey, D.W., 1995. Daily selection of feeding areas by cattle in homogeneous and heterogeneous environments. *Applied Animal Behaviour Science*, 45, pp.183–200.

Bailey, D.W., Rittenhouse, L.R., Hart, R.H., Swift, D.M., Richards, R.W., 1989. Association of relative food availabilities and locations by cattle. *Journal of Range Management*, 42, pp.480–482.

Bassett, L., Buchanan-Smith, H.M., 2007. Effects of predictability on the welfare of captive animals. *Applied Animal Behaviour Science*, 102, pp.223–245.

Bateson, P., 1979. Do sensitive periods arise and what are they for? *Animal Behaviour*, 27, pp.470–486.

Bergsten, C., Pettersson, B., 1992. The cleanliness of cows tied in stalls and the health of their hooves as influenced by the use of electric trainers. *Preventive Veterinary Medicine*, 13, pp.229–238.

Bewley, J.M., Boyce, R.E., Hockin, J., Munksgaard, L., Eicher, S.D., Einstein, M.E., Schutz, M.M., 2010. Influence of milk yield, stage of lactation, and body condition on dairy cattle lying behaviour measured using an automated activity monitoring sensor. *The Journal of Dairy Research*, 77, pp.1–6.

Bishop-Hurley, G.J. Swain, D., Anderson, D.M., Sikka, P., Crossman, C., Corke, P., 2007. Virtual fencing applications : Implementing and testing an automated cattle control system. *Computers and Electronics in Agriculture*, 56, pp.14–22.

Bloomsmith, M.A., Jones, M.L., Snyder, R.J., Singer, R.A., Gardner, W.A., Liu, S.C., Maple, T.L., 2003. Positive reinforcement training to elicit voluntary movement of two giant pandas throughout their enclosure. *Zoo Biology*, 22, pp.323–334.

Boissy, A., Terlouw, C., Le Neindre, P., 1998. Presence of cues from stressed conspecifics increases reactivity to aversive events in cattle: evidence for the existence of alarm substances in urine. *Physiology & Behavior*, 63, pp.489–495.

Brantas, G.C., 1968. Training, eliminative behaviour and resting behaviour of Friesian-Dutch cows in a cafeteria stable. *Zeitschrift für Tierzüchtung und Züchtungsbiologie*, 85, pp.64–77.

Broom, D.M., 1991. Animal welfare: concepts and measurement. *Journal of Animal Science*, pp.4167–4175.

Coblentz, B., 1976. Functions of scent marking in ungulates with special reference to feral goats (Capra hiricus L.). *The American Naturalist*, 110, pp.549–557.

Colavita, F.B., 1974. Human sensory dominance. *Perception & Psychophysics*, 16, pp.409–412.

Conrad, C.D., 2010. A critical review of chronic stress effects on spatial learning and memory. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, 34, pp.742–755.

Cook, N.B., 2009. Free-stall Comfort Design for Maximum Cow. In *WCDS Advances in Dairy Technology*. pp. 255–268.

Cooper, M.D., Arney, D.R., Phillips, C.J.C., 2010. The motivation of high- and low-yielding dairy cows to obtain supplementary concentrate feed. *Journal of Veterinary Behavior: Clinical Applications and Research*, 5, pp.75–81.

Coulon, M., Deputte, B.L., Heyman, Y., Baudoin, C., 2009. Individual recognition in domestic cattle (Bos taurus): evidence from 2D-images of heads from different breeds. *PloS one*, 4, p.e4441.

Coulon, M., Baudoin, C., Heyman, Y., Deputte, B.L., 2011. Cattle discriminate between familiar and unfamiliar conspecifics by using only head visual cues. *Animal Cognition*, 14, pp.279–290.

Daros, R.R., Costa, J.H.C., von Keyserlingk, M.A.G., Hotzel, M.J., Weary, D.M., 2013. Individual housing impairs reversal learning in dairy calves. In *International Society for Applied Ethology*. p. 88.

de Passillé, A.M., Rushen, J., Ladewig, J., Petherick, C., 1996. Dairy calves' discrimination of people based on previous handling. *Journal of Animal Science,* 74, pp.969–974.

de Paula Vieira, A., de Passillé, A M., Weary, D.M., 2012. Effects of the early social environment on behavioral responses of dairy calves to novel events. *Journal of Dairy Science*, 95, pp.5149–55.

Duff, G.C., Galyean, M.L., 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. *Journal of Animal Science*, 85, pp.823–840.

Entsu, S., Dohi, H., Yamada, A., 1992. Visual acuity of cattle determined by the method of discrimination learning. *Animal Behaviour*, 34, pp.1–10.

Erina, S., Cziszter, L.T., Acatinc, S., Baul, S., Tripon, I., George, G., Gavojdian, D., 2012. Study on the Excretion Behaviour in Romanian Black and White Primiparous Cows . Number of Defecations. *Animal Science and Biotechnologies*, 45, pp.316–319.

Ekesbo, I., 2011. *Farm Animal Behaviour: Characteristics for Assessment of Health and Welfare*, CABI.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., Jones, R.B., Meuniersalaun, M., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, 92, pp.340–374.

Fregonesi, J., Veira, D.M., von Keyserlingk, M.A.G., Weary, D.M., 2007. Effects of bedding quality on lying behavior of dairy cows. *Journal of Dairy Science*, 90, pp.5468–5472.

Friend, T.H., 1991. Behavioral aspects of stress. *Journal of Dairy Science*, 74, pp.292–303.

Fujiwara, M., Rushen, J., de Passillé, A.M., 2014. Dairy calves adaptation to group housing with automated feeders. *Applied Animal Behaviour Science*, 158, pp.1–7.

Gaillard, C., Meagher, R.K., von Keyserlingk, M.A.G., Weary, D.M., 2014. Social housing improves dairy calves' performance in two cognitive tests. *PloS one*, 9, p.e90205.

Grandin, T., Odde, K.G., Schutz, D.N., Behrns, L.M., 1994. The reluctance of cattle to change a learned choice may confound preference tests. *Applied Animal Behaviour Science*, 39, pp.21–28.

Grandin, T., Rooney, M. B., Phillips, M., Cambre, R. C., Irlbeck, N. A., Graffam, W., 1995. Conditioning of nyala (Tragelaphus angasi) to blood sampling in a crate with positive reinforcement. *Zoo Biology*, 14, pp.261–273.

Hafez, E.S.E., Schein, M.W., 1962. The behaviour of cattle. In E. S. E. Hazef, ed. *The Behaviour of Domestic Animals*. London: Bailliere Tindall and Cox.

Hagen, K., Broom, D.M., 2003. Cattle discriminate between individual familiar herd members in a learning experiment. *Applied Animal Behaviour Science*, 82, pp.13–28.

Hall, C.A., Cassaday, H.J., Derrington, A.M., 2003. The effect of stimulus height on visual discrimination in horses. *Journal of Animal Science*, 81, pp.1715–1720.

Heinonen-tanski, H., Mohaibes, M., Karinen, P., Koivunen, J., 2006. Methods to reduce pathogen microorganisms in manure. *Livestock Science*, 102, pp.248 – 255.

Hespos, S.J., Baillargeon, R., 2008. Young infants' actions reveal their developing knowledge of support variables: converging evidence for violation-of-expectation findings. *Cognition*, 107, pp.304–316.

Hirata, M., Higashiyama, M., Hasegawa, N., 2011. Diurnal pattern of excretion in grazing cattle. *Livestock Science*, 142, pp.23–32.

Holm, L., Jensen, M.B., Jeppesen, L.L., 2002. Calves' motivation for access to two different types of social contact measured by operant conditioning. *Applied Animal Behaviour Science*, 79, pp.175–194.

Hopster, H., van der Werf, J.T.N., Blokhuis, H.J., 1998. Side preference of dairy cows in the milking parlour and its effects on behaviour and heart rate during milking. *Applied Animal Behaviour Science*, 55, pp.213–229.

Howery, L., 2013. Potential for using visual, auditory and olfactory cues to manage foraging behaviour and spatial distribution of rangeland livestock. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 8, pp.1–10.

Hultgren, J., 1991. A preliminary study of behavioural methods for assessing the influence of electric cow-trainers on animal health. *Veterinary Research Communications*, 15, pp.291–300.

Hultgren, J., Bergsten, C., 2001. Effects of a rubber-slatted flooring system on cleanliness and foot health in tied dairy cows. *Preventive Veterinary Medicine*, 52, pp.89–75.

Jago, J., Kerrisk, K., 2011. Training methods for introducing cows to a pasture-based automatic milking system. *Applied Animal Behaviour Science*, 131, pp.79–85.

Kovalcik, K., Kovalcik, M., 1986. Learning ability and memory testing in cattle of different ages. *Applied Animal Behaviour Science*, 15, pp.27–29.

Krohn, C.C., Munksgaard, L., 1993. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments II. Lying and lying-down behaviour. *Applied Animal Behaviour Science*, 37, pp.1–16.

Laca, E.A., 1998. Spatial memory and food searching mechanisms of cattle. *Journal of Range Management*, 51, pp.370–378.

Langbein, J., Siebert, K., Nurnberg, G., 2009. On the use of an automated learning device by group-housed dwarf goats : Do goats seek cognitive challenges? *Applied Animal Behaviour Science*, 120, pp.150–158.

Lauber, M.C.Y., Hemsworth, P.H., Barnett, J.L., 2006. The effects of age and experience on behavioural development in dairy calves. *Applied Animal Behaviour Science*, 99, pp.41–52.

Lee, C., Prayaga, K., Reed, M., Henshall, J., 2007. Methods of training cattle to avoid a location using electrical cues. *Applied Animal Behaviour Science*, 108, pp.229–238.

Lowe, D.E., Steen, R.W.J., Beattie, V.E., 2001. Preferences of housed finishing beef cattle for different floor types. *Animal Welfare*, 10, pp.395–404.

Magdesian, K.G., Williams, D.C., Aleman, M., Lecouteur, R.A., Madigan, J.E., 2009. Evaluation of deafness in American Paint Horses by phenotype, brainstem auditory-evoked responses, and endothelin receptor B genotype. *Journal of the American Veterinary Medical Association*, 235, pp.1204–1211.

Maier, S.F., Seligman, M.E., 1976. Learned helplessness: Theory and evidence. *Journal of Experimental Psychology: General*, 105, pp.3–46.

McCall, C. a. et al., 2003. Equine utilization of a previously learned visual stimulus to solve a novel task. *Applied Animal Behaviour Science*, 82, pp.163–172.

Müller, C.A., Mayer, C., Dörrenberg, S., Huber, L., Range, F., 2011. Female but not male dogs respond to a size constancy violation. *Biology Letters*, 7, pp.10–13.

Munksgaard, L., de Passillé, A.M., Rushen, J., Thodberg, K., Jensen, M.B., 1997. Discrimination of People by Dairy Cows Based on Handling. *Journal of Dairy Science*, 80, pp.1106–1112.

Oltenacu, P., Hultgren, J., Algers, B., 1998. Associations between use of electric cow-trainers and clinical diseases, reproductive performance and culling in Swedish dairy cattle. *Preventive Veterinary Medicine*, 37, pp.77–90.

Oudshoorn, F.W., Kristensen, T., Shahrak, E., 2008. Dairy cow defecation and urination frequency and spatial distribution in relation to time-limited grazing. *Livestock Science*, 113, pp.62–73.

Pajor, E.A., Rushen, J., de Passillé, A.M., 2003. Dairy cattle’s choice of handling treatments in a Y-maze. *Applied Animal Behaviour Science*, 80, pp.93–107.

Pell, A.N., 1997. Manure and Microbes : Public and Animal Health Problem? *Journal of Dairy Science*, 80, pp.2673–2681.

Phillips, C.J.C., Lomas, C.A., 2001. The perception of color by cattle and its influence on behavior. *Journal of Dairy Science*, 84, pp.807–813.

Platt, S., Freeman, J., di Stefani, A., Wieczorek, L., Henley, W., 2006. Prevalence of unilateral and bilateral deafness in border collies and association with phenotype. *Journal of Veterinary Internal Medicine*, 20, pp.1355–1362.

Prescott, N., Mottram, T., Webster, A. J., 1998. Relative motivations of dairy cows to be milked or fed in a Y-maze and an automatic milking system. *Applied Animal Behaviour Science*, 57, pp.23–33.

Price, K., 1972. Predictable and unpredictable shock: their pathological effects on restrained and unrestrained rats. *Pyschological Reports*, 30, pp.419–426.

Rehkämper, G., Perrey, A., Werner, C.W., Opfermann-Rüngeler, C., Görlach, A., 2000. Visual perception and stimulus orientation in cattle. *Vision Research*, 40, pp.2489–2497.

Renken, W.J., Howery, L.D., Ruyle, G.B., Enns, R.M., 2008. Cattle generalise visual cues from the pen to the field to select initial feeding patches. *Applied Animal Behaviour Science*, 109, pp.128–140.

Roth, L.S.V., Lind, O., 2013. The impact of domestication on the chicken optical apparatus. *PloS one*, 8, p.e65509.

Rushen, J., de Passillé, A.M., Borderas, F.,Tucker, C.,Weary, D., 2004. Designing Better Environments for Cows to Walk and Stand. *Advances in Dairy Technology*, 16, pp.55–64.

Rushen, J., Munksgaard, L., Marnet, P.G., DePassillé, A.M., 2001. Human contact and the effects of acute stress on cows at milking. *Applied Animal Behaviour Science*, 73, pp.1–14.

Sahara, D. Ichikawa, T., Aihara, Y., Kaanishi, H., & Nagashima, M., 1990. Eliminative and reposeing behavior of dairy cows in the stanchion stall barn. *Japanese Journal of Zootechnical Science*, 61, pp.249–254.

Saitoh, T., Seo, T., Kashiwamura, F., 2005. Control of cattle defecation in resting area using feeding station. In *Proceedings of the 39th International Congress of the International Society for Applied Ethology*. Kanagawa, Japan: ISAE 2005, p. 127.

Santos, L.R., Hauser, M.D., 1999. How monkeys see the eyes: cotton-top tamarins' reaction to changes in visual attention and action. *Animal Cognition*, 2, pp.131–139.

Schaeffer, R.G., Sikes, J.D., 1971. Discrimination learning in dairy calves. *Journal of dairy science*, 54, pp.893–896.

Schrijver, N.C.A., Pallier, P.N., Brown, V.J., Würbel, H., 2004. Double dissociation of social and environmental stimulation on spatial learning and reversal learning in rats. *Behavioural Brain Research*, 152, pp.307–314.

Simon, T.J., Hespos, S.J., Rochat, P., 1995. Do infants understand simple arithmetic? A replication of Wynn (1992). *Cognitive Development*, 10, pp.253–269.

Somers, J.G.C.J., Frankena, K., Noordhuizen-Stassen, E.N., Metz, J.H.M., 2003. Prevalence of claw disorders in Dutch dairy cows exposed to several floor systems. *Journal of Dairy Science*, 86, pp.2082–2093.

Stefanowska, J., Tiliopoulos, N.S., Ipema, A.H., Hendriks, M.M.W.B., 1999. Dairy cow interactions with an automatic milking system starting with `walk-through' selection. *Applied Animal Behaviour Science*, 63, pp.177–193.

Telezhenko, E., Bergsten, C., 2005. Influence of floor type on the locomotion of dairy cows. *Applied Animal Behaviour Science*, 93, pp.183–197.

Telezhenko, E., Lidfors, L., Bergsten, C., 2007. Dairy cow preferences for soft or hard flooring when standing or walking. *Journal of dairy science*, 90, pp.3716–3724.

Terlouw, E.M.C., Boissy, A., Blinet, P., 1998. Behavioural responses of cattle to the odours of blood and urine from conspecifics and to the odour of faeces from carnivores. *Applied Animal Behaviour Science*, 57, pp.9–21.

Tongeľ, P., Brouček, J., 2010. Influence of hygienic condition on prevalence of mastitis and lameness in dairy cows. *Slovak Journal of Animal Science*, 43, pp.95–99.

Tucker, C.B., Weary, D.M., de Passillé, A.M., Campbell, B., Rushen, J., 2006. Flooring in Front of the Feed Bunk Affects Feeding Behavior and Use of Freestalls by Dairy Cows. *Journal of Dairy Science*, 89, pp.2065–2071.

Uetake, K., Hurnik, J.F., Johnson, L., 1997. Effect of music on voluntary approach of dairy cows to an automatic milking system. *Applied Animal Behaviour Science*, 53, pp.175–182.

Uetake, K., Kudo, Y., 1994. Visual dominance over hearing in feed acquisition procedure of cattle. *Applied Animal Behaviour Science*, 42, pp.1–9.

Umstatter, C., Brocklehurst, S., Ross, D.W., Haskell, M.J., 2013. Can the location of cattle be managed using broadcast audio cues? *Applied Animal Behaviour Science*, 147, pp.34–42.

Vaddella, V.K., Ndegwa, P.M., Joo, H.S., Ullman, J.L., 2010. Impact of Separating Dairy Cattle Excretions on Ammonia Emissions. *Journal of Environment Quality*, 39, p.1807.

Villettaz Robichaud, M., de Passillé, A.M., Pellerin, D., Rushen, J., 2011. When and where do dairy cows defecate and urinate? *Journal of Dairy Science*, 94, pp.4889–4896.

Wang, S.H., Baillargeon, R., Brueckner, L., 2004. Young infants' reasoning about hidden objects: evidence from violation-of-expectation tasks with test trials only. *Cognition*, 93, pp.167–198.

Watts, J.M., Stookey, J.M., 2000. Vocal behaviour in cattle : the animal's commentary on its biological processes and welfare. *Applied Animal Behaviour Science*, 67, pp.15–33.

Waynert, D.F., Stookey, J.M., Watts, J.M., Waltz, C.S., 1999. The response of beef cattle to noise during handling. *Heart*, 62, pp.27–42.

Whistance, L.K., Arney, D.R., Sinclair, L.A., Phillips, C.J.C., 2007. Defaecation behaviour of dairy cows housed in straw yards or cubicle systems. *Applied Animal Behaviour Science*, 105, pp.14–25.

Whistance, L.K., 2009. Eliminative behaviour of dairy cows. *Core Organic project Series*, pp.10–22.

Whistance, L.K., Sinclair, L.A., Arney, D.R., Phillips, C.J.C., 2011. Eliminative behaviour of dairy cows at pasture. *Applied Animal Behaviour Science*, 130, pp.73–80.

Whistance, L.K., Sinclair, L.A., Arney, D.R., Phillips, C.J.C., Richard, D., 2009. Trainability of eliminative behaviour in dairy heifers using a secondary reinforcer. *Applied Animal Behaviour Science*, 117, pp.128–136.

Willis, G.L., Mein, G., 1982. Classical conditioning of milk ejection using a novel conditioned stimulus. *Applied Animal Ethology*, 9, pp.231–237.

Wredle, E., Rushen, J., de Passillé, A.M., Munksgaard, L., 2004. Training cattle to approach a feed source in response to auditory signals. *Canadian Journal of Animal Science*, 84, pp.567–572.

Wredle, E., Munksgaard, L., Sporndly, E., 2006. Training cows to approach the milking unit in response to acoustic signals in an automatic milking system during the grazing season. *Applied Animal Behaviour Science*, 101, pp.27–39.

Yerkes, R.M., Dodson, J.D., 1908. The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparitive Neurology and Psychology*, 18, pp.459–482.

Zurbrigg, K., Kelton, D., Anderson, N., Millman, S., 2005. Tie-stall design and its relationship to lameness, injury, and cleanliness on 317 Ontario dairy farms. *Journal of Dairy Science*, 88, pp.3201–3210.

2. Rationale and objectives

Accumulation of urine and faeces in dairy barns is a risk factor for many human and cattle health problems and also has negative environmental impacts. Current attempts to control where cattle urinate and defecate rely on building designs which restrict cattle's natural behaviour. Cattle have excellent spatial memory and have been successfully trained to associate urination and defecation with a food reward. It may be possible to train cattle to urinate and defecate in specific areas, expediting collection and processing of manure and reducing contamination.

Before a training protocol to control where cattle urinate and defecate can be developed and implemented on farm, a number of details of their urination defecation habits and learning abilities must be identified. This thesis does not attempt to address all elements of training ability, nor is it a comprehensive review of urination or defecation habits of cattle. Instead it focuses on areas that may have practical application and also contributes to our basic understanding of the challenge in manipulating behaviour associated with urination and defecation. This thesis also helps pave the way for needed future work in this area.

The objectives of this thesis are:

1) To determine the frequency of urination or defecation by group housed calves.
2) To identify the locations and times urination and defecation by group housed calves most frequently occurs.
3) To identify if calves are capable of forming an association between a specific location/context and urination.
4) To determine the relative effectiveness of classical or operant conditioning techniques for urination training.
5) To determine if urination training can be recalled when cattle are retested after a few months in a new location.
6) To examine the effectiveness of classical conditioning to improve calves attention to colour cues and generalising these cues to a new context.
7) To identify and classify learning errors in a Y maze colour discrimination task.

3. Urination and defecation by group housed dairy calves

This chapter represents the first step in developing a training protocol for urination training. Within this chapter the urination and defecation patterns of group housed calves was recorded.

Vaughan, A., Marie de Passillé, A., Stookey, J., Rushen, J. (2014). Urination and defecation by group-housed dairy calves. *Journal of dairy science*. 97 pp. 4405-4411

This manuscript was originally drafted by Alison Vaughan with suggested comments from Drs. Anne Marie de Passillé, Jeffrey Rushen and Joseph Stookey. Experimental design, data collection and analysis was under taken by Alison Vaughan with advice from Dr. Jeffrey Rushen and Anne Marie de Passillé.

3.1 Abstract

A better understanding of when and where group housed calves are most likely to defecate or urinate might permit improved housing design or more efficient use of cleaning routines. Despite this, this is the first study to address the urination and defecation habits of calves. The primary aims of this study were to report the daily frequency of calves' urination and defecation and determine when and where group housed dairy calves defecate and urinate most frequently. We were also interested to see if incidence of urination and defecation changed with increasing age and the change in diet at weaning. We observed 36 female Holstein calves housed in groups of nine, and fed milk, grain, and hay from automated feeders. For the purposes of another experiment, these calves were assigned to one of three experimental treatments relating to age at start of weaning and milk allowance; low milk allowance and early weaning (6 L/d, 42 d), high milk allowance and early weaning (12 L, 42 d), high milk allowance and late weaning (12 L, 84 d) The occurrence of defecations and urinations was determined by continuous observation of video-recordings taken over 72h at two age periods (Age; mean ± sd, Period 1 = 32.0 ± 11.13 d and Period 2 = 61 ± 11.29 d). Due to the treatments, weaned and unweaned calves were observed in each period (period 1; 34 unweaned and 2 weaned calves; period 2; 16 unweaned and 20 weaned calves). Large differences were found between calves in mean daily frequency of total urinations and defecations across 3 d period (mean = 17.56 ± 5.07/d, range = 4.33 to 28.67). Differences between individual calves did not change significantly over time, provided calves remained unweaned. Two days of observation was sufficient to give a reliable estimate of daily urination and defecation frequency. Frequency of urination and defecations was higher in calves post-weaning. Higher age and visits to the milk feeder were associated with a higher frequency of urinations and defecations, pre-weaning. After weaning, frequency of eliminations increased with increasing visits to the water feeder. There was an effect of time of day with significantly more events during daylight hours (06:00-18:00 h) in comparison to night (18:00 h-06:00 h). Before weaning, calves urinated and defecated significantly more on slatted flooring and sawdust bedded areas than within the feeder (daily mean ± sd = 6.96 ± 3.15, 6.49 ± 3.90 and 4.10 ± 2.67 for slatted floor, bedded floor and feeder areas, respectively). Frequency of eliminations in feeders and slatted, but not sawdust bedded, areas was higher in calves post-weaning. Calves urinate and defecate more frequently during daylight hours when they are more active. Slatted

flooring around feeders is useful to reduce soiling of bedded areas, particularly as calves increase in age.

Keywords: dairy calf, urination, defecation

3.2 Introduction

There is increasing interest in housing unweaned dairy calves in groups, which has the potential to reduce the labor associated with both cleaning and feeding (Kung et al., 1997). Accumulation of the faeces and urine of group-housed calves can increase the potential for transmission of disease between conspecifics and also pose a risk to human health (Pell, 1997). Furthermore, the release of volatile ammonia, occurring when urea (found in urine) comes into contact with urease (found in faeces), is related to several environmental problems (Moreira & Satter 2006; Sheppard et al., 2007). Despite the importance of elimination behaviors, little is known of the factors that influence defecation or urination by cattle and, to our knowledge, no studies have addressed the urination and defecation habits of calves. A better understanding of when and where group housed calves defecate and urinate might permit improved housing design, reduced soiling of bedding or more efficient use of cleaning routines.

Some studies have suggested a link between diet and frequency of defecations but this link has not yet been supported by evidence (Hirata et al., 2011; Villettaz Robichaud et al., 2011). The majority of studies have examined urination and defecation in adult dairy cattle, which are typically subject to intensive management practices with relatively rigid daily routines. Although Villettaz Robichaud et al. (2011) found no diurnal rhythm in urination and defecation behaviors nor any correlation between the frequency of urination and defecation in each hour of the day and feeding activity, Aland et al. (2002) found that most defecation in adult cows occurred during the hours when the animals were most active; that is, during milking and feeding. Group housed calves fed milk and grain ad libitum via automated feeders are not subject to an artificially imposed time budget and it would be interesting to see if a similar pattern of activity in elimination behaviors is seen.

Some studies have recorded the locations where cows were most likely to defecate or urinate (Whistance et al., 2007; Oudshoorn et al., 2008; Villettaz Robichaud et al., 2011). Cattle permitted limited access to pasture were found to defecate and urinate over their entire grazing surface, without accumulation in specific “hot spots” (Oudshoorn et al., 2008). In contrast, in free stall housing urination and defecation was concentrated in feed alleys and alleyways behind the stalls which may reflect how much time the cows spent in that area (Whistance et al., 2007; Villettaz Robichaud et al., 2011).

The aims of this study were to determine when and where group housed dairy calves defecate and urinate most frequently, and to look at the relationship between the frequency of defecation and urination by calves, pre- and post-weaning, and in relation to age, feed intake, feeder visits, weaning and time of day.

3.3 Materials and methods

Thirty-six female Holstein calves (mean ± sd birth weight = 43.12 ± 4.74 kg) were removed from their dams and fed 4 L of high quality colostrum within 6 h of birth and housed individually in concrete floored pens (1.22 m x 2.44 m) with sawdust bedding. Within the first 24 h of age, calves were weighed and an identification tag fitted with a transponder attached to the left ear. During this period they received 12 L of pasteurized whole milk per d (i.e. *ad libitum*) in two meals (approximately 08:00 h and 15:00 h) via an artificial teat attached to the pen wall.

At 4-5 d of age (mean = 4.5 d), calves were added to group pens until there were nine calves within each pen, with a maximum age range of 30 d within each group. Each group of nine calves was housed in a pen with a 4.74m x 2.48m plastic coated, expanded metal floor at the front of the pen and a 4.74m x 4.64m concrete-based area, bedded with wood shavings at the rear (Fig 3.1.). Fresh bedding was added to each pen once per week.

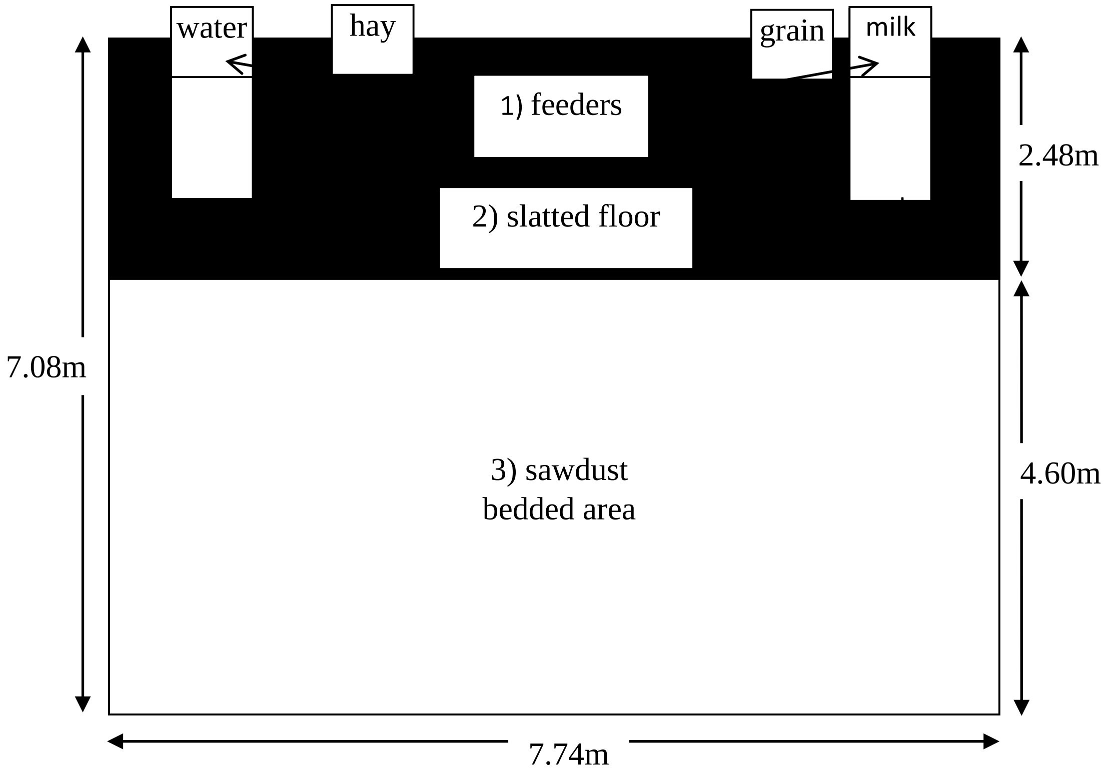


Figure 3.1. - Layout of experimental calf pen showing the three locations; 1) feeders, 2) slatted floor and 3) sawdust bedded area.

The pens contained one automated milk feeder and one automated grain feeder (DeLaval CF 1000 CS Combi, Sweden), one automated hay feeder and one automated drinker (Insentec, Marknesse, Holland). The automated milk feeder, situated at the front of each group pen, provided calves with filtered and pasteurized waste milk via an artificial teat. Portions of milk became available from the feeder throughout the day and could either be consumed as each new portion became available or accumulated across several hours (e.g. for an allowance of 12 L / d, an additional 0.5 L would become available every hour, up to a maximum of 6 L in one visit to the feeder). Grain feeders dispensed commercial calf starter mix (17.4% protein, 6.37% fiber and 4.38% fat; Unifeed Ltd, Chilliwack, Canada) in 20 g portions, at a maximum rate of 9 kg per d (i.e. *ad libitum*). Grass hay (DM = 90.8%, CP = 15.1%, NDF = 51.1%, and ADF = 33.6%) and water were freely available. For the purposes of another experiment (de Passillé et al., 2011), calves entering the group pen were assigned to one of three experimental treatments relating to age at start of weaning and milk allowance; low milk allowance and early weaning (6 L / d, 42 d), high milk allowance and early weaning (12 L, 42 d), high milk allowance and late weaning (12 L, 84 d). Three calves from each treatment were in each group pen.

DeLaval feeders measured the daily individual milk and grain intakes for each calf using the volume of feed dispensed. Both grain and milk feeders recorded the number and duration of visits. The hay feeders and drinkers were equipped with hydraulic scales which allowed for number of visits and consumption of hay and water to be measured. Visits to feeders without consumption of feed were excluded from analyses. Data from milk and grain feeders were recorded and stored by Kalbmanager and Win_Institute programs (Foerster-Technik, Engen, Germany). Water and hay consumption was recorded by Insentec RIC – System IV TIRIS Identification Roughage/Water Version 11 UH7802 (Insentec, Marknesse, The Netherlands).

3.3.1. Behavioral observations

Three overhead video cameras (Panasonic, WVBP 334; Oskaka, Japan) were mounted on the ceiling above each pen, and an additional camera was mounted so that the entire pen could be viewed and recorded continuously at normal speed using digital video recorders (Genetec Inc., Saint-Laurent, QC, Canada). The videos were read at four times normal speed. To validate video identification of elimination behaviours, 4 h of direct observation were compared with overhead video.

The four groups of calves were randomly allocated between three observers. Each group of calves was watched by a single trained observer. All observers completed training before beginning data collection using a sample video. Following three repetitions of the sample video, inter and intra-observer reliability were calculated using the results. Experimental data collection only commenced once the reliability was of a satisfactory level ($r_{Pearson} > 0.8$.). This process was repeated, using the same footage, halfway through watching each group to ensure data collection remained consistent.

To obtain a range of ages and milk allowances, each group of calves was watched at two periods, four weeks apart. To ensure data reflected a good representation of the calves' behavior, three consecutive d (3x24 h) were selected for each observation period, with a total of six d per calf. Due to the weaning treatments (n = 12 calves per treatment), weaning occurred at different ages and thus both weaned and unweaned calves were observed at each observation period (Observation period 1; 34 unweaned and 2 weaned calves; Observation period 2; 16 unweaned and 20 weaned calves). On each observation day all the calves within a group pen were watched and the type (urination/defecation/unidentified), time (to the nearest second) and location of each elimination event was recorded for each calf. The location of the calf during defecation and urination was recorded using three locations as shown in Fig. 3.1. A defecation or urination event was considered to have occurred in the feeder if the calf's head was within the feeder stall. If the calf was moving, the location where the first drop of urine or faeces fell was recorded. The position of calves (e.g. standing or lying) was also noted.

Eliminations were identified using the characteristic posture adopted by calves (defecation: tail lifted and back slightly arched; urination: tail lifted, back arched, hind legs placed forward and apart) and/or evidence of either manure (new faeces on the floor) or urine (spray of urine on the floor) (Villettaz Robichaud et al., 2011). In some incidences it was not possible to distinguish the type of elimination event from the video and these events that could not be confidently categorized were marked as "unidentified". To determine whether urinations and defecations were equally likely to be unidentified a sample of 4 h of direct and video observation of urinations and defecations were compared.

3.3.2. Organization of data and analysis

Data were analyzed using SAS software (SAS Institute Inc, Cary, NY, USA). Data was reported for individual calves within group pens. For each calf, the mean daily frequencies of defecation, urination, unidentified events and total events were calculated for each three d observation period, and the distributions of these data were described using the minimum, 25th quartile, median, 75th quartile and maximum. As it was not possible to distinguish between urination and defecation in 28% of the events, unidentified events were combined with urinations and defecations to give total eliminations for all subsequent analysis

There was no difference between weaning treatments in the frequency of eliminations (student t test: T = 0.22, df =11, P = 0.83) so treatments were pooled for all subsequent analysis. The quantities of milk, starter feed, hay, and water consumed by each calf were automatically recorded by feeders but a full data set was available for only 33 calves. As calves were weaned at different ages due to the weaning treatments, we had unequal numbers of pre and post weaning data within each observation period. Data were available for 31 pre weaning calves (age range:

17-58 d) in observation period 1, for 20 post weaning calves in observation period 2 (age range: 56-82 d) and both pre and post weaning data were available for 18 of the calves.

Pearson correlation was calculated between the mean daily frequency of urinations, defecations and unidentified events (pooled) and age, milk, grain, hay and water intakes and frequency of visits to feeders across each 3 d observation period for pre ($n = 31$) and post weaning ($n = 20$) periods. Stepwise regression analysis was conducted to determine which of the independent variables (age, milk, grain, hay and water intakes and feeder visits) had the greatest influence on frequency of eliminations, pre and post-weaning. Paired t tests were used to test for differences in frequency of events between night and day and frequency of events between pre and post. Differences in the frequency of eliminations between the 3 different locations were tested using a one way ANOVA with pairwise comparisons of mean values using Tukey's test.

To determine how many days of observation are required to give a reliable estimate of daily elimination frequency of calves 1d, 2d and 3d means were compared using Pearson correlation within and between each period. Pearson correlation was also used to compare three d averages between observation periods; calves which were pre-weaned in both observation periods ($n = 16$) and calves weaned between observation periods ($n = 18$) were considered separately. The frequency distribution of eliminations across 24 h was compared between pre and post weaning using Pearson correlation.

3.4. Results

Large differences were found between calves in the frequency of elimination events (Table 3.1.). Comparison between the 4 h of direct and video observations revealed that defecations and urinations were not equally likely to be uncategorized, with the latter accounting for almost 2/3 of unidentified events. Differences in the frequency of eliminations between individuals were relatively consistent between the two observation periods for calves which had not begun the weaning process ($r = 0.84$, $df = 14$, $P < 0.001$) but for calves which were weaned between the two observation periods no significant correlation in the frequency of elimination was found ($r = 0.29$, $df = 14$, $P = 0.24$). Calves were never observed to urinate or defecate while lying down.

Table 3.1. – Daily frequency of elimination events averaged across the two observation periods

	Event type	Minimum	25th percentile	Median	75th percentile	Maximum
All observations (n = 36)	Defecation	0.33	3.67	5.33	8.00	14.67
	Urination	1.33	5.33	6.33	8.50	13.67
	Unidentified	1.67	3.67	4.33	5.92	12.33
	Total eliminations	4.33	14.67	17.33	21.00	28.67
Pre weaning (n = 34)	Defecation	0.33	3.33	4.50	5.67	9
	Urination	1.33	5.33	6.33	7.67	12.67
	Unidentified	1.67	3.33	4.33	5.33	12.33
	Total eliminations	4.33	13.33	15.5	18.67	28.33
Post-weaning (n = 20)	Defecation	4.33	6.67	9.00	10.00	14.67
	Urination	2.67	4.83	6.50	9.17	13.67
	Unidentified	2.67	4.00	4.83	7.67	12.00
	Total eliminations	14.67	18.00	21.83	23.33	28.67

Calves had significantly more elimination events after weaning (paired student t test; T = 4.59, df = 17, P < 0.001: mean ± sd, pre = 17.33 ± 2.16 and post = 21.33 ± 3.83), consequently pre- and post-weaning calves were considered separately in subsequent analysis. Before weaning, a significant low correlation was found between frequency of elimination events and age, and a tendency towards a positive correlation with visits to the water and milk feeders and overall feeder visits was found (Table 3.2.). Multiple linear regression of pre-weaning data found that age and frequency of visits to the milk feeder were the main factors explaining variation in frequency of eliminations (n = 31, age; F = 8.35, P = 0.01, visits to milk feeder; F = 5.06, P = 0.03). After weaning, eliminations were correlated with frequency of visits to the water feeder and a non-significant trend was found for eliminations to increase with increasing water intake (Table 3.2.). The results of regression analysis, for post weaning data, indicated that frequency of visits to the water feeder was the only variable accounting for variation in frequency of eliminations (n = 20, F = 4.53, P = 0.05).

Table 3.2. – Pearson correlations between frequency of urinations and defecations (pooled) and age, grain, hay, water and milk intake and feeder visits across 36 h, for pre- and post-weaning observations.

	Mean frequency of urinations and defecations (across 36 h)	
	Pre weaning (n = 31)	Post weaning (n = 20)
Age	0.49 P = 0.01	0.24 P = 0.32
All feeder visits	0.33 P = 0.07	0.20 P = 0.41
Grain intake	0.05 P = 0.78	0.36 P = 0.12
Visits to grain feeder	0.13 P = 0.48	0.03 P = 0.90
Hay intake	0.02 P = 0.90	-0.08 P = 0.74
Visits to hay feeder	0.05 P = 0.78	-0.08 P = 0.73
Water intake	0.30 P = 0.10	0.43 P = 0.06
Visits to drinker	0.35 P = 0.05	0.45 P = 0.05
Milk intake	0.24 P = 0.19	n/a
Visits to milk feeder	0.32 P = 0.08	n/a

We found some evidence of diurnal rhythms in the frequency of elimination events (Figure 3.2.). There were significantly more events during daylight hours (07:00–18:00 h) in comparison to night (19:00–06:00 h) (student t test: T = 22.65, df = 35, P < 0.001; mean: 10.73 and 1.79 events for day and night, respectively). The fewest events occurred between 04:00-05:00 h and frequency of eliminations sharply increased at 07:00 h, peaking at 08:00 h. The frequency distribution of eliminations across 24 h did not change significantly after weaning (r = 0.69, df = 34, P < 0.001). Frequency of calves' eliminations per h and the frequency of visits to the feeders per h were highly correlated (Figure 3.2.; r = 0.86, df = 22, P < 0.001).

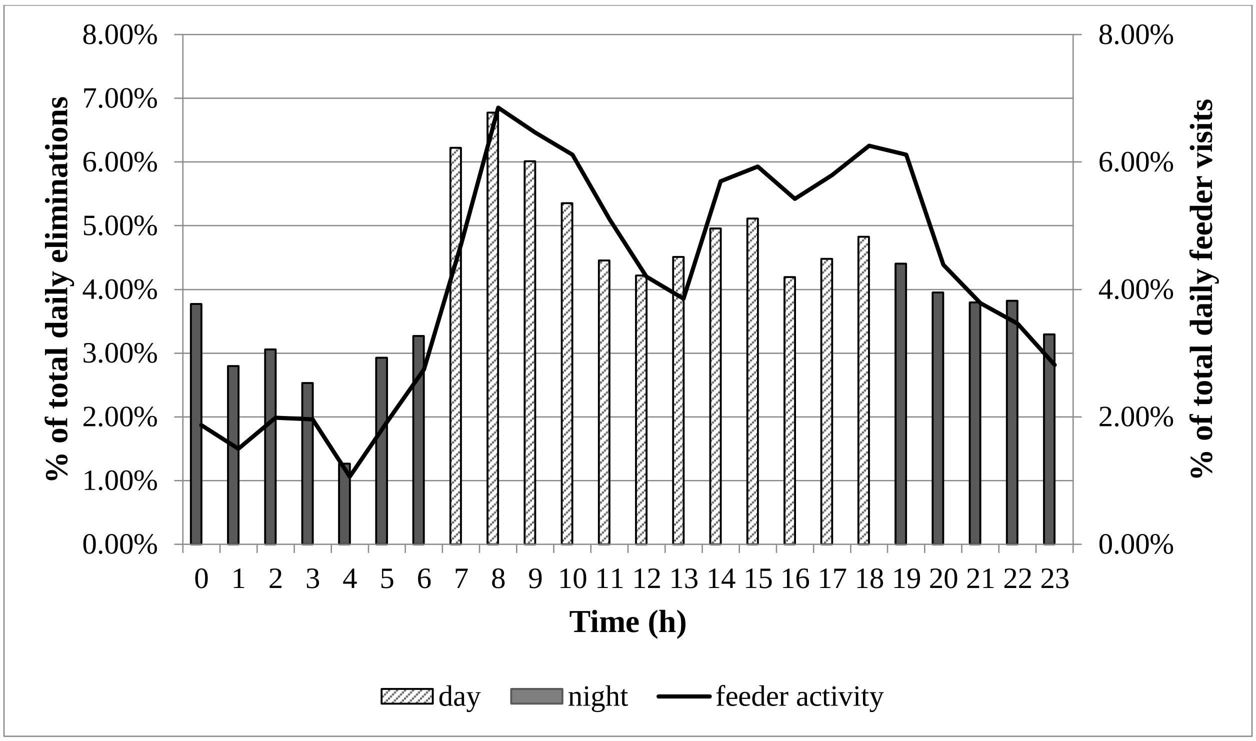


Figure 3.2. - Percentage of the daily elimination events (urination and defecation pooled) and percentage of total daily visits to the feeders occurring at each hour of the day. Data averaged across 6 d, n = 36.

There was no difference in the frequency of elimination events occurring on the slatted floor and bedded sawdust areas, although significantly less events occurred within feeders than other areas (One way ANOVA; n = 36, F (2,33) = 7.88, P = 0.001; mean frequency ± sd = 6.96 ± 3.15, 6.49 ± 3.90 and 4.10 ± 2.67 for slatted floor, bedded floor and feeder areas, respectively).

After weaning, there was a significant increase in the frequency of urinations and defecations occurring in feeders and the slatted area of the group pen (paired t test: T = 4.12, df= 17, P = 0.01 and 2.69, P = 0.02, respectively) but no significant change in the frequency of urinations and defecations on the sawdust bedded area (paired t test: T = 0.51, df =17, P = 0.61; Fig. 3.3.).

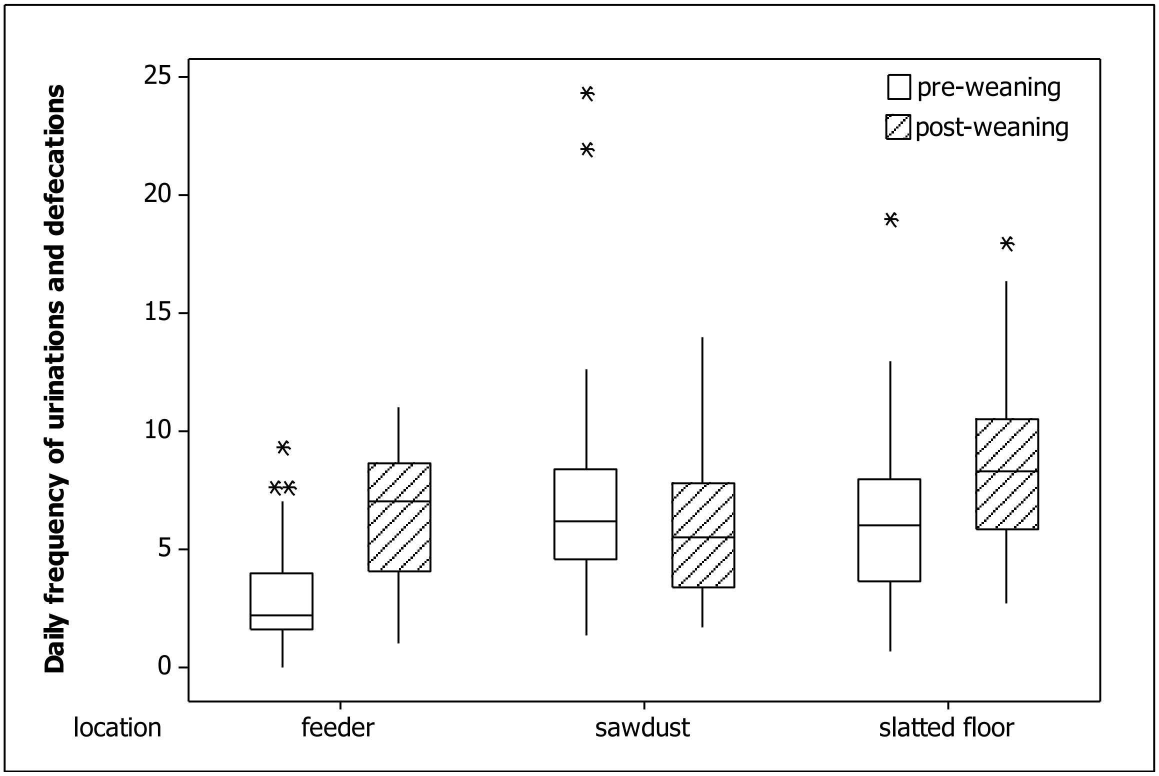


Figure 3.3. – Daily frequency of urination and defecation occurring in each location within the pen, pre- and post-weaning (n = 18). The box plot shows the median and 25th and 75th percentile of daily frequency of eliminations (urinations and defecations pooled), lines show min and max values, asterisks show outliers.

At each period, there was a low to moderate correlation between calves' frequency of elimination on individual days. Moderate to strong correlations were observed between one d and three d averages (pre: r = 0.86, df = 32, P < 0.001; post: r = 0.77, df = 18, P < 0.001). Two day averages however were highly correlated both with individual day (pre: n = 34, r = 0.92, df = 32, P < 0.001; post: r = 0.82, df = 18, P < 0.001) and 3 d averages (pre: r = 0.97, df = 32, P < 0.001; post: r = 0.95, df = 18, P < 0.001).

3.5. Discussion

There were large differences in the frequency of eliminations between individual calves, something which has also been noted in studies of adult cattle (Villettaz Robichaud et al., 2011). Day-to-day variation was observed in the frequency of individual calves' eliminations, however observing two consecutive days was sufficient to give an accurate estimation of daily frequency. Interestingly, the frequency of eliminations was highly correlated between the two observation periods, one month apart, but only for calves which were not weaned between these periods.

The frequency of urination and defecation increased significantly after weaning. Prior to weaning, age and frequency of visits to the milk feeder were the main factors accounting for the variation in frequency of eliminations between calves. After weaning, frequency of eliminations was positively correlated with visits to the water feeder. Interestingly, frequency of visits to the feeder appeared to be more important than amount of feed consumed. As it was not possible to confidently categorize all incidences of urination and defecation, it is not clear whether the increase in daily eliminations represents an increase in urinations or defecations.

Even taking into account uncategorized eliminations (the greater proportion of unidentified events were found to be urinations when video was compared with direct observations), daily frequency of observed defecations was approximately half that reported for cows kept in tie-stall barns and in free stall and straw-pen barns (Aland et al., 2002; Whistance et al., 2007; Villettaz Robichaud et al., 2011). Given the shift from a mainly liquid to solid feed occurring at weaning, it seems intuitive that an increase in the frequency of defecations would account for the observed increase in daily eliminations post weaning. However, both before and after weaning frequency of eliminations was correlated with visits to milk and water feeders and no correlation was found between either solid feed intake or visits to hay or grain feeders.

A link between feed intake and frequency of defecation has been proposed by other studies (Hirata et al., 2011). However the variation in the frequency of defecation and urination was not related to variation in feed and water intake in Villettaz Robichaud et al. (2011) and it may be that the increase in frequency of events owes more to increasing age. Aland et al. (2002) reported possible age effects on urination and defecation behavior, with cows defecating but not urinating more frequently than heifers. In the current study older calves had a higher frequency of eliminations prior to weaning but this relationship was not found in post weaning observations. The age range of calves observed prior to weaning was greater than that of those observed post weaning and it may be that an age effect would also be apparent after weaning had the variation in age been greater between observed calves.

We did not measure the volume of urine and faeces excreted in the current study and it may be that diet had an effect on the volume of urine and faeces excreted at each episode. To our knowledge, although attempts have been made to measure faecal output/defecation (Hirata et al., 2011), no study has validated frequency of urinations and defecations as a measure of volume of urine and faeces excreted. It may be that differences in volume/elimination event accounts for some of the large individual variation reported between adult cattle in Villettaz Robichaud et al. (2011). Nevertheless, frequency of eliminations is likely a more important measure in terms of cleanliness, since a calf which defecates frequently may be more likely to spread manure around than one which defecates larger volumes in fewer locations.

We found a significant difference in the frequency of eliminations between day and night, with significantly more events during 06:00-18:00 h, when calves have been reported to be more active (Hänninen et al., 2005). In the current study the frequency of urinations and defecations per h was strongly correlated with feeding behavior (as estimated by frequency of feeder visits per h). The diurnal pattern was correlated between pre and post-weaning periods and did not appear to be influenced by age or total daily frequency of eliminations. The proportion of elimination events occurring per hour bears a striking similarity to that reported both for cattle on pasture (Hirata et al., 2011) and in ties stalls (Aland et al., 2002). In contrast, Villettaz Robichaud et al. (2011) reported no diurnal pattern in either defecation or urination behaviors, although a large proportion of defecations and eliminations occurred directly after cows changed from lying to standing (also reported in Aland et al., 2002) which supports the suggestion that change in activity from resting to active behaviors may lead to an increase in the frequency of events.

There was no significant difference between the mean daily frequency of events occurring on the sawdust bedded area and the slatted area around the feeders. The frequency of eliminations events around the slatted floor and feeders, but not sawdust bedded area, increased significantly with increasing age. This may reflect calves spending more time in and around the feeders, particularly directly after weaning when visits to the milk feeder increase dramatically as calves unsuccessfully attempt to access milk (de Passillé et al., 2011). Additionally, this may reflect a change in the activity budget, and as a result time spent in different areas of the pen, as time spent resting decreases with increasing age with a particular drop around weaning (Hänninen et al., 2005). Using slatted flooring around feeders has the potential to keep group pens cleaner, particularly as calves become more active with increasing age and during weaning.

3.6 References

Aland, A., Lidfors, L., Ekesbo, I., 2002. Diurnal distribution of dairy cow defecation and urination. *Applied Animal Behaviour Science*. 78, pp.43–54.

de Passillé, A.M., Borderas, T.F., Rushen, J., 2011. Weaning age of calves fed a high milk allowance by automated feeders: effects on feed, water, and energy intake, behavioral signs of hunger, and weight gains. *Journal of Dairy Science*. 94, pp.1401–1408.

Hänninen, L., de Passillé, A. M., Rushen, J., 2005. The effect of flooring type and social grouping on the rest and growth of dairy calves. *Applied Animal Behaviour Science*. 91, pp.193-204.

Hirata, M., Higashiyama, M., Hasegawa, N., 2011. Diurnal pattern of excretion in grazing cattle. *Livestock Science*. 142, pp.23–32.

Kung, L. Jr., Demarco, S., Siebenson, L. N., Joyner, E., Haenlein, G. F., Morris, R. M., 1997. An evaluation of two management systems for rearing calves fed milk replacer. *Journal of Dairy Science*. 80, pp.2529-2533.

Moreira, V. R., Satter, L. D., 2006. Effect of scraping frequency in a freestall barn on volatile nitrogen loss from dairy manure. *Dairy Science*. 89, pp.2579–2587.

Oudshoorn, F. W., Kristensen, T., Shahrak, E., 2008. Dairy cow defecation and urination frequency and spatial distribution in relation to time-limited grazing. *Livestock Science*. 113, pp.62–73.

Pell, A., 1997. Manure and microbes: Public and animal health problem? Journal of Dairy Science. 80, pp.2673-2681.

SAS Institute. 2011. SAS User's Guide. SAS Institute Inc., Cary. NC

Sheppard, S. C., S. Bittman, J. Tait, S. J. Sommer, J. Webb., 2007. Sensitivity analysis of alternative model structures for an indicator of ammonia emissions from agriculture. *Canadian Journal of Soil Science*. 87, pp.129–139.

Villettaz Robichaud, M., Passillé, A. M. D., Pellerin, D., Rushen, J., 2011. When and where do dairy cows defecate and urinate? *Journal of Dairy Science*. 94, pp.4889–4896.

Whistance, L. K., Arney, D. R., Sinclair, L. A., Phillips, C. J. C., 2007. Defecation behaviour of dairy cows housed in straw yards or cubicle systems. *Applied Animal Behaviour Science*. 105, pp.14–25.

3.7. Conclusions and link to next chapter

The first step in addressing whether cattle can be trained to urinate and defecate in specific locations is to determine the urination and defecation habits of cattle, such as when and where cattle urinate and defecate. For example, the daily frequency of urination and defecation dictates the number of opportunities to reinforce eliminations occurring in a target area. While the elimination behaviours of adult dairy cows are well documented, the urination and defecation habits of calves have never been previously reported. The urination and defecation frequency of calves fell within the range of those reported for adult cattle. There was a large amount of individual variation in urination and defecation frequency. As with adult cattle, these differences appeared to be consistent across time but this was only true if calves were not weaned from milk between observations.

The following chapter reports the results of two methods for building an association between being placed in a target location and urinating**.** This is a key step in identifying the feasibility of training cattle to eliminate in specific areas. Calves were chosen as age of animal has been identified as a factor influencing training success; younger animals typically display more behavioural flexibility and greater performance in learning tasks, plus their size was more conducive to handling and environmental manipulations. Identifying the daily frequency, and particularly the individual variation, of urination by calves was important for designing the training protocols described in the next chapter. The times at which peak urination and defecation frequency were observed were used to identify optimal time for urination training sessions.

The next chapter explores the use of both classical conditioning and operant conditioning to train milk-fed dairy calves to urinate when placed in a stall. Classical conditioning relies on repeated pairings of a previously neutral cue (entering the stall) and an involuntary behaviour (in this case urination induced by diuretic) to build an association. In operant conditioning, an association between a cue (entering the stall) and a voluntary behaviour (urination in stall) is reinforced, in this case by release from the stall and access to a milk reward. Failure to urinate in the allotted time was punished using a “time out”. The efficacy of these two methods is described.

4. Operant conditioning of urination by calves

This chapter presents an exploration of two training methods used to build an association between a specific location and urination by dairy calves. Both classical and operant conditioning techniques employed to train calves. The efficacy of both of these methods was reported.

Chapter 4 has been published and is reproduced here with the permission of the copyright owner (Applied Behaviour Science).

Vaughan, A., de Passillé, A. M., Stookey, J., Rushen, J. (2014). Operant conditioning of urination by calves. *Applied Animal Behaviour Science*, 158, 8-15.

This manuscript was originally drafted by Alison Vaughan with suggested comments from Drs. Anne Marie de Passillé, Jeffrey Rushen and Joseph Stookey. Experimental design, animal handling, data collection and analysis was under taken by Alison Vaughan with advice from Dr. Jeffrey Rushen and Anne Marie de Passillé.

4.1. Abstract

The accumulation of faeces and urine in dairy barns is a cause of cattle and human health concerns and environmental problems. It is usually assumed that cattle are not capable of controlling defecation and urination. We tested whether calves could be taught to urinate in a location using either classical or operant conditioning. Twenty-four female Holstein calves were alternately assigned as treatment or control (Experiment 1: n = 12, median age, range = 39, 31-50 d; Experiment 2: n = 12, median age, range = 50, 29-64 d). Experiment 1 used classical conditioning, involving repeated pairing of entry into a stall and injection of a diuretic. During the training period (d 1-5) treatment calves were repeatedly placed in the stall (150 x 45 x 120 cm) and injected IV with diuretic (Salix, Intervet Inc. at 0.5 mL / kg BW) to induce urination. During the test period (d 6-15) calves were held in the stall for 10 min without diuretic injection, and urinations, defecations and vocalisations were recorded. The procedure was identical for control calves except saline was used in place of a diuretic. In the test period, the classically conditioned calves did not urinate more than controls (means ± SE: 4.3 ± 1.28 vs. 6.0 ± 1.41, for treatment and control calves, respectively). In Experiment 2, calves were trained using operant conditioning. On training days, operant calves were placed in the stall, received IV of diuretic (Salix, Intervet Inc. at 0.5 mL / kg BW) and, upon urination, were released from the stall to receive approx. 250 mL milk reward. On test days, calves were placed in the stall but did not receive diuretic; calves that urinated received the milk reward but calves failing to urinate within 15 min were given 5 min "time out" and received diuretic the following day. Yoked controls were never given diuretic but held in the stall for the same amount of time and received the same "reward" or "punishment" as their matched operant calf the previous day. Urinations, defecations and vocalisations occurring in the stall on test days were compared between treatment calves and controls. Calves trained using operant conditioning had a higher frequency of urinations in the stall than their controls (means ± SE = 5.25 ± 0.95 vs. 2.32 ± 0.52). The results of our experiment show it may be feasible to train cattle to urinate in specific areas using operant conditioning.

Keywords: calf, learning, operant conditioning, training, urination

4.2. Introduction

The accumulation of faeces and urine in dairy barns leads to poor cow hygiene, mastitis and lameness, which reduce the welfare and productivity of the cows (Hultgren and Bergsten, 2001; Reneau et al., 2005). Cow faeces can contain infectious bacteria, posing a risk to human health, and volatile emissions released when urine and faeces mix result in environmental problems (Bittman and Mikkelsen, 2009). These risks can be reduced by minimizing the spread of faeces within the barn and improving waste management. Cattle defecate between 3-29 / d and urinate 2-20 / d, producing approx. 30 kg faeces and 15 kg urine daily (Aland et al., 2002; Hirata et al., 2011; Villettaz Robichaud et al., 2011). Defecation and urination also occur when cattle are stressed, in conjunction with other behavioural measures such as vocalisation (Kilgour, 1975; Lauber et al., 2006).

Electric cow trainers are commonly used in tie-stall barns to prevent the stalls from becoming dirty by training cows to take a step backwards before urinating or defecating to avoid an electric shock (Bergsten and Pettersson, 1992). However, the use of electric trainers has been associated with an increased risk of silent heat, ketosis, mastitis (Oltenacu et al., 1998), hock injuries and reduced cleanliness (Zurbrigg et al., 2005). Future attempts to control where cattle urinate and defecate should explore alternative methods that avoid these negative impacts.

Cattle are often considered to have little voluntary control of urination and defecation (Whistance et al., 2009) but cows have excellent spatial memory (Bailey et al., 1989), and may be able to learn to eliminate in specific locations (Whistance, 2009). Simple operant conditioning techniques have been successfully employed to collect urine from mares used in the PMU (pregnant mare urine) industry (McCartney et al., personal communication, 2011) and it may be possible to adapt this method to cattle. Whistance et al., (2009) explored whether dairy heifers could be trained to control their eliminative behavior using operant conditioning. First, they trained heifers to expect a food reward after urinating or defecating, and then they attempted to ‘shape’ this behaviour to a specific area of the pen, rewarding urinations and defecation only when they occurred in the desired location. While the heifers learned to approach the trainer before and immediately after urinating or defecating to claim their food reward, it was not possible to train heifers to eliminate in a specific area of the pen.

The first step towards developing a successful training protocol is to establish if it is possible for cattle to learn to urinate or defecate within specific locations. In this study, we chose to study urination as this can be easily and rapidly stimulated artificially with diuretics. The added benefit of using a diuretic is that a single dosage induces many urinations within a short space of time, allowing many opportunities to build an association and reducing the inter-trial interval (the time between trials). We used younger calves than Whistance et al., (2009) since these may be more easily trained and handled.

We examined whether classical conditioning (repeatedly pairing a particular location with urination induced by a diuretic), and operant conditioning (where urination in the stall was rewarded) could be used to increase the frequency of urination in a location.

4.3. Materials and Methods

This study was conducted at the UBC Dairy Education and Research Centre in Agassiz, BC, Canada. All experimental conditions and procedures met the requirements of the Canadian Council for Animal Care.

4.3.1. Experimental animals

Twenty four female Holstein calves were assigned as treatment or control based on birth order (Experiment 1: n = 12, median age = 39 d, range = 31-50 d; Experiment 2: n = 12, median age = 50 d, range 29-64 d). Calves were housed individually until 5-6 d of age at which point they were moved to a group pen (9 calves per pen). Here they were provided with a milk allowance of 12 L / d (i.e. *ad libitum*) via an automated milk feeder (DeLaval® CF 1000 CS Combi, Tumba, Sweden). Calves remained in group pens for the duration of the experiment and were only removed to take part in training and testing sessions. These sessions took place within the same barn in an identical pen which housed the experimental apparatus (Fig 4.1.). For training and testing sessions calves were taken individually from their group pen to the experimental pen, where they were visually but not audibly isolated from other calves. None of the calves had taken part in a training experiment prior to this.

4.3.2. Experiment 1 – Classical conditioning

Two days before beginning the experimental phase, all calves were brought individually to the experimental pen and walked through the holding stall (Fig. 4.1.) without stopping, in order to familiarise them with the stall and the experimental set up. This process was repeated 2 x per d over 2 d. The experiment was divided into a training period (d 3-7) and a test period (d 8-15). During the training period, training sessions occurred once a day, Monday-Friday, beginning at approximately 08:00 h. An observer began recording all urinations occurring in the calves' home pen 30 min prior to the beginning of a training or testing session, and continued observations throughout. Only calves which had not urinated in the previous 30 min were brought for training. Classically conditioned and control calves were trained or tested on the same day, always beginning with a control calf and alternating between training and control calves thereafter. Entry order to the stall was recorded.

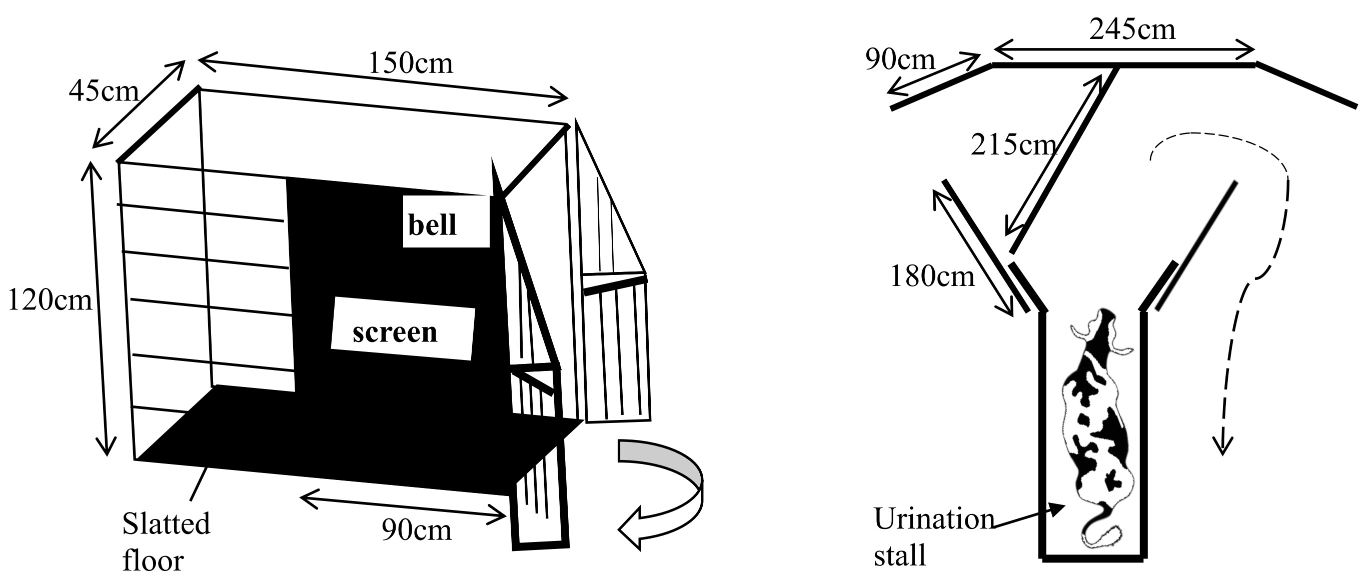


Figure 4.1. - (a) Urination stall (used in Experiments 1 and 2) (b) Set up for Experiment 1

Calves in the classical conditioning treatment were placed in the stall, a halter was used to hold the calves' head up to expose the jugular vein and calves were injected IV with a diuretic, Furosemide (Salix, Intervet Inc., Kirkland, QC, Canada at 0.5 mL / kg BW). As soon as the diuretic was injected the handler removed the halter and moved out of sight. Calves remained in the stall for a set time (10 min) to allow time for the diuretic to act. Upon release calves were returned to the stall for an additional 10 min, regardless of whether they had urinated during the first ten min in the stall, to allow an additional opportunity for urination following entry to the stall. This process was repeated over 5 d, giving training calves ten potential pairings of entering the stall and urinating during the training period. A minimum of eight urinations was required to progress to the testing period. An additional day of training was given to calves not meeting this criterion and, if they did not urinate on this additional day, they were excluded from the experiment. The procedure was identical for control calves, except that the same volume of saline was used in place of the diuretic.

During the subsequent test period, calves were individually placed in the stall and held for 10 min without any injection. Urinations within the stall were recorded. Defecations and vocalisations were also recorded as a measure of stress (Kilgour, 1975; Lauber et al., 2006).

4.3.3. Experiment 2 – Operant conditioning

The week before training began the calves' milk allowance from the automatic feeder was gradually reduced from 12 L to 9 L / d. The day before the urination training phase of this experiment began this was further reduced to 6 L and remained at this allowance until the end of the experiment. Access to the milk feeder was blocked one h prior to testing/training. This was done to increase the reward value of milk used as a reinforcer in this experiment.

During the three days prior to the experimental phase, all calves were walked through the urination stall (Fig 4.2.a) without stopping and exited either to the right, where they received a 250 mL milk reward paired with a bell (the secondary reinforcer) (Fig 4.2.; a), or to the left, where they received a 5 min "time out" with no milk reward (Fig 4.2.; b). This process was repeated 3x per session for each exit, with 1 session per d for 3 d for each calf. The first time calves exited on the reward side a handler guided them to the teat to access the milk reward. To prevent calves learning an alternating pattern of reward and time out, the order in which individual calves were walked through the experimental set up (i.e. reward side or punishment side) was determined according to a 'Gellermann series' (Gellermann, 1933) that was modified to be used with sequences of six events.

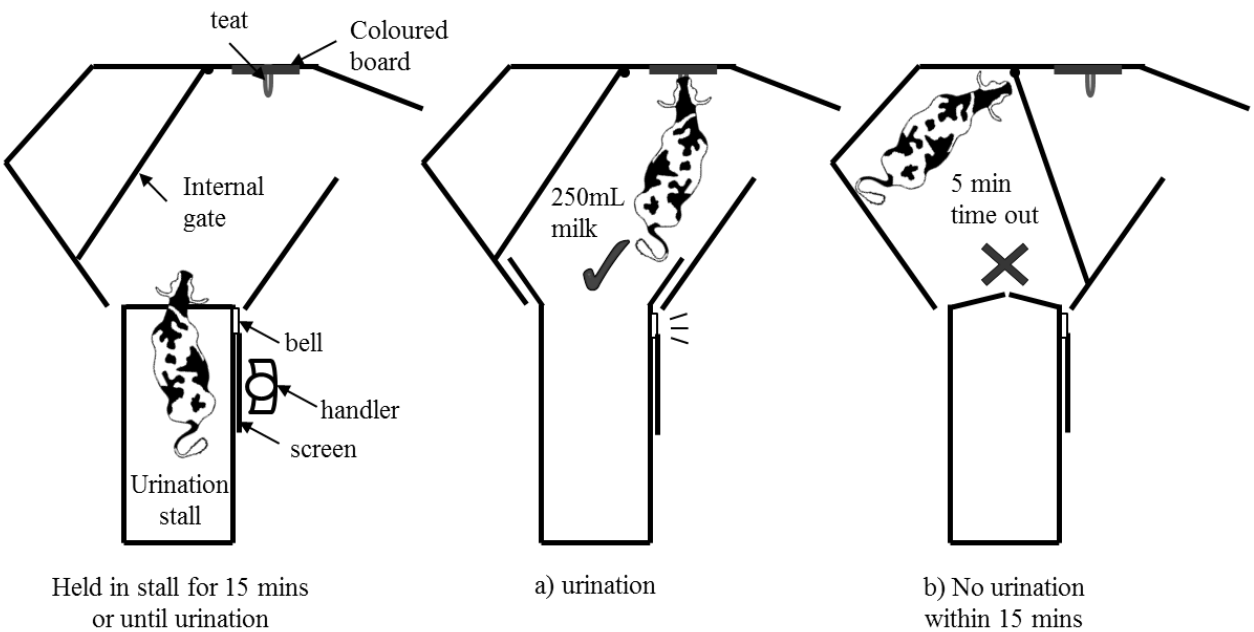


Figure 4.2. – Set up for operant calves on test days (same dimensions as Experiment 1).

Calves were assigned as operant or control calves on the basis of birth order until there were six pairs of operant and control calves. For calves in the operant treatment, the reward (access to milk) or punishment (the "time out") was given depending on whether they urinated or not whereas the yoked control calves were subjected to the same conditions as their partners, irrespective of whether they urinated in the stall or not.

On "training" days, operant calves were moved individually from their home pen and guided into the urination stall (Fig 4.1.; a). As in Experiment 1, they were restrained to receive an IV injection of diuretic, Furosemide (Salix, Intervet Inc. Kirkland, QC, Canada at 0.5 mL / kg BW), and remained in the stall until they urinated. When the calves urinated, the bell sounded and calves were immediately released from the stall to the right, where they could receive a 250 mL milk reward via a teat mounted in the rear wall. Once the calf had finished drinking the milk, she was guided back into the stall and this process was repeated two more times, such that each training day consisted of three pairings of urination and reward. Following IV diuretic injection calves have an increased frequency of urination, and second and third urinations reliably occurred within minutes of the first. The first day was a training day and all training days were always followed with a test day.

On “test” days operant calves were moved into the urination stall and allowed 15 min to urinate. If the calves urinated within 15 min, the secondary-reinforcing bell was sounded and the calf was released from the stall to receive their milk reward (Fig 4.2.; a). If an operant calf failed to urinate within 15 min the internal gate was moved and the calf exited into a small time-out pen without food where it was held for 5 min (b). If the operant conditioned calves urinated within 15 min during a test day, the following day was a test day. If they failed to urinate within the allotted time, the following day was a training day.

For both training days and test days, the yoked control calves were held in the stall for the same amount of time and exited the stall through the same side (i.e. reward or punishment) as their matched operant calves had been the previous day.

Calves were trained over 17 d (+ 2 d habituation) with one session per day (beginning at approximately 08:00 h). On the first day of training, only operant calves were put through the experimental protocol described previously. On subsequent days all calves were tested on the same day, alternating between control and operant calves. As in Experiment 1, an observer recorded all urinations in the home pen and calves were brought for training only if they had not urinated in their home pens during the previous 30 min. Entry order to the stall was recorded.

Time of each of the following events was recorded for each calf: entry to stall, injection of diuretic (where applicable) and urination. The number of defecations and vocalisations of both yoked control and treatment calves within the stall was also recorded.

4.3.4. Statistical analysis

Statistical analysis was carried out using Minitab Statistical Software 16.0 (Minitab, State College, PA, USA). Student *t*-tests were used to compare the number of urinations, defecations and vocalisations occurring on test days between treatment and control groups in Experiment 1. Paired *t*-tests were used for comparisons between operant calves and their yoked controls in Experiment 2.

Control and trained calves entered the stall in alternating order in case recent urination in the stall was likely to induce urination in subsequent calves. Effect of urination by the previous calf on the likelihood of urination by the next calf entering the stall was further examined using a Chi Squared Test.

Control calves in Experiment 1 received a saline injection whereas those in Experiment 2 did not. To examine if the saline IV injection alone increased the frequency of urination, a student *t*-test was used to compare the frequency of urinations on training days between those control calves which received IV injection of saline (Experiment 1) and those which did not (Experiment 2).

4.4. Results

4.4.1. Experiment 1

During the training period, calves receiving diuretic urinated significantly more than controls ($P < 0.001$, df = 5, T = 6.17, student *t*-test; means ± SE: treatment = 9.83 ± 2.14 and control = 3.60 ± 1.14 urinations in stall during training days). Mean latency to first urination following injection of diuretic was 4 min 23 s (range = 15 s to 10 min). Five out of six calves in training treatment reached the criterion of eight urinations in ten stall visits that was necessary to pass to the testing stage (Table 4.1.). The calf not reaching criterion received an additional day of training and continued to the test period after achieving ten urinations in 12 stall visits.

Table 4.1. - The frequency of urinations in training and test periods for treatment calves and control calves in Experiment 1.

Treatment calf	**Training (d3-7)**	**Test (d8-15)**	**Control calf**	**Training (d3-7)**	**Test (d8-15)**
44†	8	1	43	4	9
46	13	9	45	4	8
48	8	6	47	5	5
50	11	6	49†	2	1
52	11	2	51*	9	-
56	8	2	53	3	7
Mean	9.8	4.3		4.5	6.0

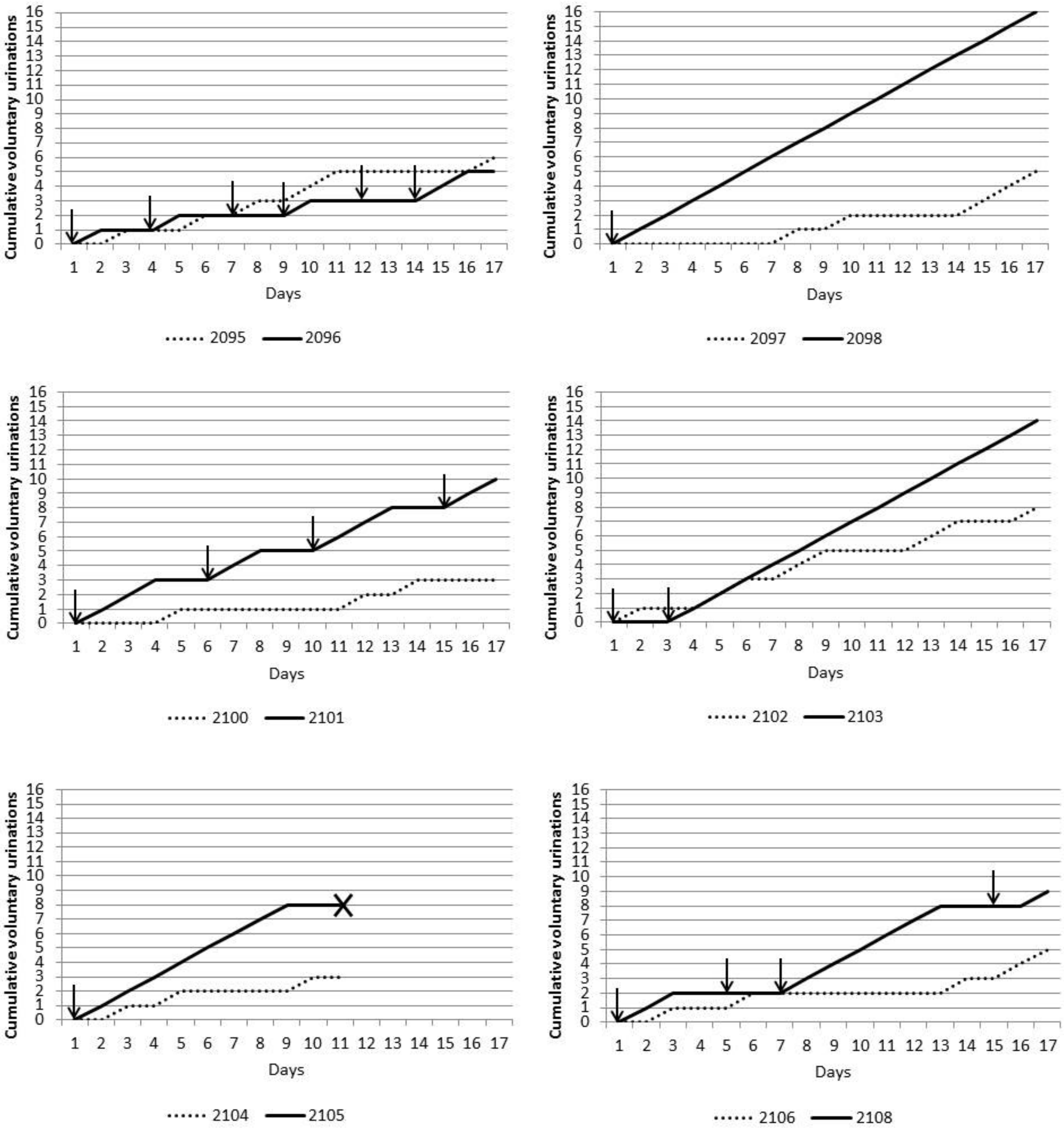
†received an extra training day

***excluded from analysis due to high frequency of urinations during training period**

There was no significant difference between treatment and control calves in frequency of urination on test days (P = 0.41, df = 5, T = 0.87, student *t*-test; means ± SE: treatment = 4.3 ± 1.28, control = 6.0 ± 1.41), the latency to urinate in the stall (P = 0.98, df = 5, T = 0.03, student *t*-test; means ± SE: treatment = 400 ± 136 s, control = 310 ± 169 s) or the frequency of vocalisation or defecation within the stall (P = 0.39, df = 5, T = 0.92 ; means ± SE: treatment = 1.26 ± 0.61, control = 3.09 ± 1.9, and P = 0.91, df = 5, T = 0.1, student *t*-test; means ± SE: treatment = 0.5 ± 0.35, control = 1.00 ± 0.45, respectively).

4.4.2. Experiment 2

The mean latency of operant calves to urinate following diuretic injection was 6 min 47 s (range = 21 s to 17 min). Operant calves urinated more frequently on test days (i.e. those not induced by diuretic) than their yoked controls (P = 0.02, df = 5, T = 3.32, paired *t*-test; means ± SE = 5.25 ± 0.95 vs. 2.32 ± 0.52 urinations). Fig. 4.3 shows the frequency of urinations on test days for each pair of calves. Five out of the six operant calves urinated more than their controls. There were however large differences between operant calves in how quickly they learned; for example #2098 urinated within the stall every test day whereas #2096 rarely urinated and was the only calf not to urinate more than her yoked control. Mean latency to urinate in the stall did not differ between operant calves and their yoked controls (P = 0.44, df = 5, T = 0.84, paired *t*-test; mean ± SE: operant = 219.89 ± 38.64 s, control = 233.25 ± 30.11 s). On test days, operant calves vocalised less than their yoked control calves (P < 0.01, df = 5, T = 2.96, paired *t*-test; means ± SE, operant = 2.52 ± 0.51, control = 5.11 ± 1.13). No difference was found in frequency of defecation within the stall between operant calves and their yoked controls (P = 0.72, df = 5T = 0.38, paired *t*-test; means ± SE, operant = 4.33 ± 1.17, control = 5.00 ± 2.02). One calf (2105) became distressed while restrained for her second injection (d 11) and, in accordance with the end points specified in the Animal Care Protocol, training/testing was discontinued at this point.



* Training of 2105 was terminated on day 11

Figure 4.3. - Cumulative number of urinations on test days for each pair of calves (urinations occurring on days when a diuretic was injected are excluded) in Experiment 2. Solid lines represent operant calves and dashed lines, their yoked control. Arrows denote injection days.

4.4.3. Effect of previous calf's urination in the stall

Urination by the previous calf was not found to influence the likelihood that the following calf would urinate in the stall (P = 0.17, df = 1, X^2=1.86).

4.4.4. Controls

Controls receiving saline injection (Experiment 1) did not urinate more on training days than those which received no injection (Experiment 2) (P = 0.39, df = 5, T = 0.92, student *t*-test; means ± SE, Experiment 1 = 0.53 ± 0.07, Experiment 2 = 0.37 ± 0.15).

4.5. Discussion

Young calves can be taught to urinate in a particular location using operant conditioning methods in which urination in the stall is associated with a food reward. However, classical conditioning methods, whereby entry into a stall is paired with urination induced by a diuretic, do not appear to be effective. Our results are in agreement with other learning studies in cattle which have found that operant but not classical conditioning was required for cattle to learn to visit a feeding station in response to a specific tone (Wredle et al., 2004).

The classical conditioning used in this experiment is an example of delayed conditioning, whereby the subject is presented with the stimulus (in this case being held in the stall) and remains exposed to the stimulus until the behavioural response is performed. The diuretic was used to reduce the delay between entering the stall and the desired behaviour (typically around 5 min). Within this experiment calves did not appear to form an association between being held in the stall and urination. This is not to say it is impossible - perhaps a higher dose of diuretic would reduce the delay between entering the stall and urination making it easier for the association to be acquired - however it does suggest that classical conditioning is not the optimal approach to toilet training cattle.

In Experiment 2, calves undergoing operant training had a higher frequency of urinations on test days (i.e. those not induced by diuretic) than their yoked controls, suggesting that they learned to associate urination with release from the stall and reward. Since yoked control calves did not receive the diuretic injections it is possible in principle that the difference arose from the repeated diuretic injections given to the operant calves. However, Experiment 1 showed that simply repeatedly pairing placement in the stall with urination was not sufficient to increase the frequency of urination in the stall, making this explanation unlikely. A second alternative explanation for the higher frequency of urination by operant calves relative to their yoked controls is that urination could have been induced by the stress of injection the previous day. Controls in the operant experiment did not receive a saline injection and thus may have been less stressed within the stall. However, frequency of urination on training days was not significantly different between controls in Experiment 1, which received saline injections, and those in Experiment 2, which did not, and thus it is unlikely that the saline injection stimulated urination in controls. Furthermore, operant calves with the best performance received the least injections, so possible stressors associated with receiving an injection seem unlikely to be responsible for the higher rate of urinations in operant calves. Another feature of conditioned responses is that the behaviour is gradually extinguished when no longer reinforced. If conditioning has occurred we see a spontaneous recovery of the conditioned task when behaviour is once more reinforced. Unfortunately, within this experiment there was not sufficient time available to permit a period of extinction and test for recovery.

The current study is the first to have shown that it is possible to train young calves to urinate using operant conditioning. A small number of studies have attempted to stimulate cattle to urinate and defecate in specific areas (Whistance et al., 2009; Villettaz Robichaud et al., 2013) and our results with young calves support those of Whistance et al. (2009), who trained older (16 month old) heifers to associate urination and defecation with a food reward.

We have not yet tested the degree to which this learning results in preferential urination in a specific location and it is possible that factors other than location (for example, handling on the way from the home pen to the stall) acted as a cue for calves. Whistance et al. (2009) attempted to shape the learned urination to a particular location, such that heifers would only be rewarded for urination or defecation in a specific area of their pen. However, the heifers did not learn to eliminate in a specific area, perhaps because the use of the floor type (concrete vs. straw) as the main cue to differentiate the target area from the rest of the pen may not have been a sufficiently salient. It may be that adding clear and specific visual cues to make the target area more distinct would increase proportion of urination and defecation events occurring in this area. Furthermore, there is anecdotal evidence to suggest that both horses and cattle preferentially urinate on soft, absorbent surfaces to avoid urine splashing on to their hind legs and this may have contributed to some individual's preference for eliminating on straw bedded rather than the target concrete floored area. An additional explanation for why heifers did not learn to urinate and defecate in the desired part of the pen in Whistance et al.'s (2009) study was that heifers were housed and trained in the same pen. As a consequence, urinating and defecating in the desired location would have been unrewarded for the majority of events, which may have interfered with their ability to learn the required task.

We found large differences between calves in their performance; while one calf (#2098) consistently urinated within 3 min of entering the stall (and consequentially only received a diuretic injection on d1), another calf (#2096) rarely urinated within the stall (Fig 4.3.). Differences in performance of the task may reflect differences in learning ability, age, temperament, motivation or physical ability. Other studies investigating learning abilities in cattle have found large variation between individuals in performance in learning tasks (Kovalcik & Kovalcik, 1986; Hagen & Broom, 2003; Whistance et al., 2009). Within the current study some variation in performance may be related to low motivation for the milk reward. Calves were fed 6 L milk /d via an automated feeder which allowed calves to drink multiple meals throughout the day and, although the feeder was disconnected one h prior to training, calves had often consumed milk prior to the disconnect. As a result some calves were not always motivated to consume the additional milk available in the reward pen and this may have significantly affected their performance. Identifying rewards which cattle are reliably motivated to access would be advantageous to future training. An effective training system must also accommodate differences in individual performance, allowing additional training sessions for animals which require it and, if possible, use multiple approaches to training.

Interestingly, operant calves vocalised significantly less than their yoked controls but no difference in the frequency of defecation was found. Defecation and vocalisation are widely used as indicators of stress (Watts & Stookey, 2000; Rushen et al., 2001). Predictability of an animal's environment is well known to reduce stress and anxiety (Bassett & Buchanan-Smith, 2007) and success in learning tasks has even been suggested to elicit a positive emotional response in cattle (Hagen & Broom, 2004). Thus the difference in vocalisation frequency may indicate that, although operant calves and their yoked controls received the same reward or punishment, the trained group experienced less stress as a result of increased control over their situation. Caution should be used however in interpreting vocalisations as indicators of positive or negative experiences without supporting evidence since the contexts in which cattle vocalise are varied (Watts & Stookey, 2000). For example, Whistance et al. (2009) noted that heifers would often vocalise when approaching to collect their food reward and it may be that vocalisation within this study could be representative of anticipation of reward rather than fear or frustration. In both studies there was no difference between treatment and control calves in the frequency of defecation within the stall. This suggests that, although more vocal in the operant training experiment, control calves were not experiencing acute stress. This is in agreement with other studies which have found cattle show signs of habituating to being handled in a specific place after three or four experiences (Waynert et al., 1999).

This study demonstrates that calves may be able to build an association between a specific location and an elimination behaviour. It would be interesting to explore the use of similar techniques to train cattle in their home pen in future studies.

4.6. Conclusion

The present study is the first study to show that young calves may be capable of forming an association between a particular location or context and performance of an eliminative behaviour. The results of this experiment show it may be feasible to train cattle to urinate in specific areas of the barn using operant conditioning.

4.7. References

Aland, A., Lidfors, L., Ekesbo, I., 2002. Diurnal distribution of dairy cow defecation and urination. *Applied Animal Behaviour Science*. 78, pp.43–54.

Bailey, D.W., Rittenhouse, L. R., Hart, R. H., Richards, R. W., 1989. Characteristics of Spatial Memory in Cattle. *Applied Animal Behaviour Science*. 23, pp.331–340.

Bassett, L. & Buchanan-smith, H.M., 2007. Effects of predictability on the welfare of captive animals. *Applied Animal Behaviour Science*. 102, pp.223–245.

Bergsten, C., Pettersson, B., 1992. The cleanliness of cows tied in stalls and the health of their hooves as influenced by the use of electric trainers. *Preventative Veterinary Medicine*. 13, pp.229–238.

Bittman, S., Mikkelsen, R., 2009. Ammonia Emissions from Agricultural Operations: Livestock. *Better Crops* 93, pp.28–31.

Gellerman, L.W., 1933. Chance orders of alternating stimuli in visual discrimination experiments. *Journal of Genetic Psychology,* 42, pp.206-208.

Hagen, K., Broom, D.M., 2003. Cattle discriminate between individual familiar herd members in a learning experiment. *Applied Animal Behaviour Science*. 82, pp.13–28.

Hagen, K., Broom, D.M., 2004. Emotional reactions to learning in cattle. *Applied Animal Behaviour Science*. 85, pp.203–213.

Hirata, M., Higashiyama, M., Hasegawa, N., 2011. Diurnal pattern of excretion in grazing cattle. *Livestock Science.* 142, pp.23–32.

Hultgren, J., Bergsten, C., 2001. Effects of a rubber-slatted flooring system on cleanliness and foot health in tied dairy cows. *Preventative Veterinary Medicine*. 52, pp.89–75.

Kilgour, B.Y.R., 1975. The open-field test as an assessment of the temperament of dairy cows. *Animal Behaviour*. 23, pp.615–624.

Kovalcik, K., Kovalcik, M., 1986. Learning ability and memory testing in cattle of different ages. *Applied Animal Behaviour Science*. 15, pp.27–29.

Lauber, M.C.Y., Hemsworth, P.H., Barnett, J.L., 2006. The effects of age and experience on behavioural development in dairy calves. *Applied Animal Behaviour Science*. 99, pp.41–52.

Oltenacu, P., Hultgren, J., Algers, B., 1998. Associations between use of electric cow-trainers and clinical diseases, reproductive performance and culling in Swedish dairy cattle. *Preventative Veterinary Medicine*. 37, pp.77–90.

Reneau, J.K., Seykora, A. J., Heins, B. J., Endres, M. I., Farnsworth, R. J., Bey, R., 2005. Association between hygiene scores and somatic cell scores in dairy cattle. *Journal of American Veterinary Medicine Association.* 227, pp.1297–1301.

Rushen, J. Munksgaard, L., Marnet, P. G., de Passillé, A. M., 2001. Human contact and the effects of acute stress on cows at milking. *Applied Animal Behaviour Science*. 73, pp.1–14.

Villettaz Robichaud, M., de Passillé, A. M., Pellerin, D., Rushen, J., 2011. When and where do dairy cows defecate and urinate? Journal of Dairy Science. 94, pp.4889–4896.

Villettaz Robichaud, M., de Passillé, A.M., Rushen, J., 2013. Does water or blowing air stimulate cows to defecate? *Applied Animal Behaviour Science*. 144, pp.98–101.

Watts, J.M., Stookey, J.M., 2000. Vocal behaviour in cattle : the animal's commentary on its biological processes and welfare. *Applied Animal Behaviour Science.* 67, pp.15–33.

Waynert, D.F., Stookey, J. M., Watts, J. M., Waltz, C. S., 1999. The response of beef cattle to noise during handling. *Heart*. 62, pp.27–42.

Whistance, L.K., Sinclair, L.A., Arney, D.R., Phillips, C.J.C., 2009. Trainability of eliminative behaviour in dairy heifers using a secondary reinforcer. *Applied Animal Behaviour Science.* 117, pp.128–136.

Wredle, E., Rushen, J., de Passillé, A.M., Munksgaard, L., 2004. Training cattle to approach a feed source in response to auditory signals. *Canadian Journal of Animal Science.* 84, pp.567–572.

Zurbrigg, K., Kelton, D., Anderson, N., Millman, S., 2005. Tie-stall design and its relationship to lameness, injury, and cleanliness on 317 Ontario dairy farms. *Journal of Dairy Science.* 88, pp.3201–3210.

4.8. Conclusions and link to next chapter

The results of this chapter suggest that calves can form an association between entering a designated area and urinating. Classical conditioning did not lead to an increase in likelihood of urination in the stall but operant conditioning techniques increased the frequency of urinations in the stall relative to controls for 5 out of the 6 calves trained. The findings of this chapter show it may be feasible to train calves to urinate in specific areas of the barn using operant conditioning.

Cattle are moved between different areas of the barn throughout their life. If training cattle to eliminate in specific places is to be successful on farm, cattle must be able to remember their training and generalise it to new locations. Thus the logical next step is to determine if the calves trained using operant conditioning in this chapter recall their training when retested in a different location, 5 months after initial training. Additionally the following chapter aims to determine if naïve heifers are able to learn via operant conditioning protocols or whether initial training has to begin at a younger age.

To test this a similar experimental set up to that described in this chapter was constructed in the barn where the heifers were housed. Attempts were made to match the set up to that described in this chapter but by necessity certain changes had to be made such as food reward offered for urinating in the stall (milk reward for unweaned calves, grain for 6 month old heifers). The performance of both naïve heifers and those which underwent operant conditioning as calves is reported.

5. Failure of operant conditioning of urination in naïve and pre-trained heifers, a short communication

This chapter presents a follow up experiment to the preceding chapter. Within this chapter the ability of heifers to recall a previously conditioned association in a new location was tested 6 months after initial training. Operant conditioning was also used to train naïve heifers of the same age to associate entering a stall and urination.

Chapter 5 is not being considered for publication.

This manuscript was originally drafted by Alison Vaughan with suggested comments from Drs. Anne Marie de Passillé, Jeffrey Rushen and Joseph Stookey. Experimental design, animal handling, data collection and analysis was under taken by Alison Vaughan with advice from Drs. Jeffrey Rushen and Anne Marie de Passillé.

5.1. Abstract

The objectives of this study were 1) to see if heifers (~ 6 months of age) can form an association between entering a stall and urination behaviour using operant conditioning and 2) whether prior training (at a younger age, in a different location and with a different reward) improved performance. Five heifers had been previously successfully trained as calves using operant conditioning to associate urinating in a stall with a milk reward. These operantly conditioned animals were retested along with their yoked controls in a similar set up (although in a different building) ~ 5 months after initial training. We also recruited 10 naïve heifers of a similar age (five heifers were trained using operant conditioning and each was assigned with a yoked control, matched for age). Heifers underwent an almost identical training/testing protocol using a diuretic to that described in Chapter 3. Operantly conditioned heifers did not urinate more than their yoked controls when tested (mean ± SE = 7.4 ± 0.92 vs 6.6 ± 0.91; matched pairs t test, $p = 0.58$). Previous training did not improve performance in the urination training task, operant heifers with prior training were no more likely than inexperienced heifers to urinate more than their controls. Further work is required to refine operant conditioning protocols to train heifers to associate entering a location with urination. Studies identifying factors which may improve cattle's abilities to generalise a learned task between different locations are also necessary.

Keywords: heifer; learning, operant conditioning, urination

5.2. Introduction

The accumulation of urine and faeces is a risk factor for human and cattle health and environmental problems (Hultgren & Bergsten 2001; Reneau et al. 2005; Bittman & Mikkelsen 2009). Cattle are commonly thought to have little control over their eliminative behaviour (Hafez & Schein 1962), consequently collection of waste is often a major feature of barn design. Unfortunately, current methods used to clean the dairy barns and reduce the contamination often restrict the behaviour of cattle and compromise cow comfort (Whistance 2009).

Training cattle to eliminate in specific locations may be an alternative solution to current cleaning methods. Operant conditioning has been successfully used to train calves to urinate upon entering a stall (Vaughan et al. 2014). It would be advantageous, and likely necessary in practical settings, for cattle to recall latrine training months later in a new location. A few studies have examined the ability of cattle to generalise a learned task to a new location, with variable success (de Passillé et al. 1996; Rybarczyk et al. 2003; Renken et al. 2008).

5.3. Materials and Methods

Heifers were divided between 4 treatments; those trained as calves in Chapter 3 and their controls and naïve heifers which were assigned to either operant conditioning or control treatments (naïve heifers; mean age = 176 ± 21.6 d). Ten of the 12 heifers used in the operant conditioning experiment described in Chapter 3 were retested five months later (five trained, five yoked controls; mean age = 199 ± 13.8 d). Two of the heifers used in Experiment 1 were excluded; one heifer assigned to the operant training protocol but which showed poor learning and one control heifer due to mortality. Heifers used in Chapter 3 were kept in their original pairings where possible. An additional ten naïve heifers, which had not been part of any training experiments prior to this, were alternately assigned as trained or yoked controls based on birth order. All heifers were housed in the same free stall pen within the heifer barn.

Two days before beginning the experimental phase heifers were given a minimum of 10 min to explore the experimental area in pairs (experimental area show in Fig 5.1.). A gate from the experimental area was opened to return heifers to their home pen only once both heifers in a pair had sampled grain from the reward bucket. The bucket was presented in a red stand made from the same material which surrounded the teat from which calves had previously received their milk reward in Chapter 3. This was done to ensure all heifers were comfortable eating from the bucket and heifers were motivated to eat the grain reward.

The following day heifers were brought individually to the experimental area and walked through the urination stall (Fig. 5.1.; a), without stopping in the stall. As in Chapter 3, a bell sounded as heifers exited to where the reward (in this Chapter, 250g of grain) was presented in the reward bucket (Fig 5.1.; b). The other exit led to a small pen where heifers received a 5 minute “time out” (Fig. 5.1.; c). Heifers were guided to each exit 3x per d for each exit, with the order determined according to a modified ‘Gellermann series’ (Gellermann, 1933). This was done to condition heifers to associate one exit with the grain reward and the other with the time out punishment.

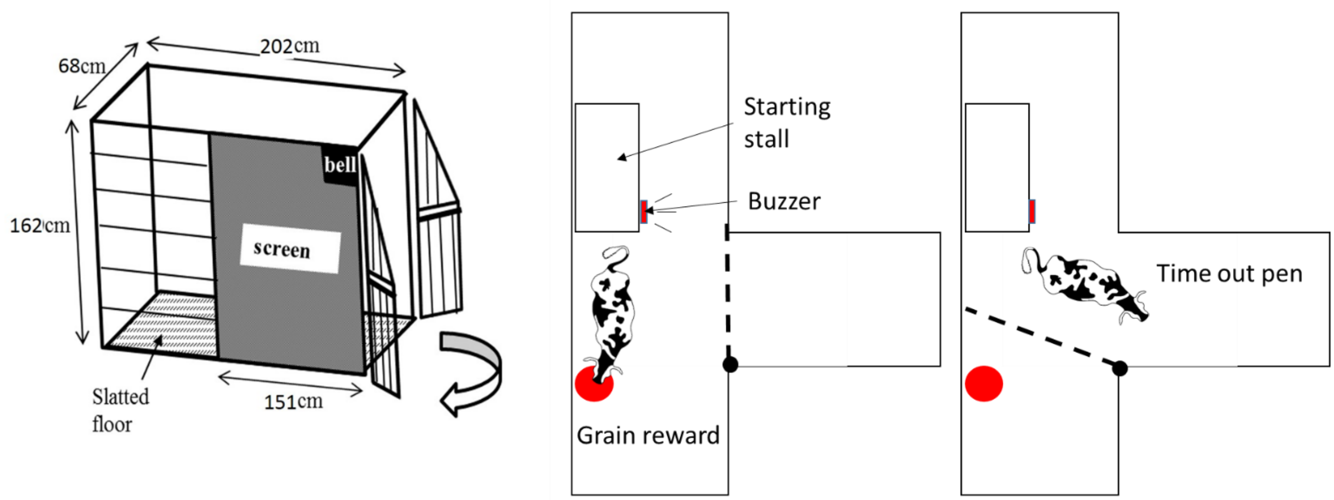


Figure 5.1. - Experimental set up; a) the starting stall b) the rewarded exit and c) the punishment exit

From then on, training and testing were conducted in an identical manner to that described for operant calves in Chapter 3, although heifers in the current study had a “rest” day every other day. Heifers received a diuretic injection on training days but not on test days. It was not possible to train/test all heifers on the same day, due to the number of animals and the time taken for each individual trial. Instead, heifers were trained/tested every other day, alternating daily between pre-trained and naïve groups.

Each heifer was trained over 15 d, with a “rest” day between each experimental day. Each experimental day heifers received only one session, beginning at approximately 0900 h. On the first day of training, only operant heifers were put through the experimental protocol described previously. On subsequent days all heifers with a group were tested on the same day, alternating between control and operant heifers. As in Chapter 3, an observer recorded all urinations in the home pen and heifers were brought for training only if they had not urinated in their home pens during the previous 30 min. Entry order to the stall was recorded.

Time of each of the following events was recorded for each heifer: entry to stall, injection of diuretic (where applicable) and urination. The number of defecations and vocalisations of both yoked control and trained heifers within the stall was also recorded.

5.3.1. Statistical Analysis

A paired t-test was used to compare the number of voluntary urinations (urinations occurring on test days) between trained heifers and their yoked controls within pre-trained and naïve treatments. Paired t-test were also used to compare the frequency of defecation and vocalisation within the stall between treatment and control heifers.

5.4. Results

Heifers in the training treatment did not have a significantly higher frequency of “voluntary urinations” (i.e. those not induced by diuretic) than their yoked controls (mean ± SE = 7.4 ± 0.92 vs 6.6 ± 0.91; matched pairs t test, $P = 0.58$). Three out of five of trained heifers urinated more than their control in both the naïve group and those with prior experience of the task (Table 5.1.).

Table 5.1. - Cumulative number of urinations across test days (urinations occurring on injection days are excluded), operant heifers and their yoked controls are shown on the same row. Those operant heifers marked in bold urinated more times (numerically) than their yoked control.

	Operant treatment		**Yoked control**	
	Heifer	Cumulative no. of urinations	Heifer	Cumulative no. of urinations
Previously trained	2108	7	2106	5
	2105	11	2104	7
	2103	7	2102	8
	2101	9	2095	7
	2098	5	2097	10
Naïve	2107	5	2093	0
	2110	11	2109	5
	2114	4	2111	9
	2118	4	2115	9
	2121	11	2119	6

Previous training did not appear to have any effect on frequency of voluntary urinations. It is not clear whether this was the result of a failure to generalise to the new location and different reward or a failure to recall previous training. A few studies have examined the ability of cattle to generalise their training to a new location but reported variable success (de Passillé et al. 1996; Rybarczyk et al. 2003; Renken et al. 2008). To the author's knowledge no work has addressed which factors may contribute to whether generalisation occurs. Cattle have been reported to possess accurate spatial memory (Collins 1989), yet most studies did not extend the period between initial training and retesting to more than 6 weeks (Kovalcik & Kovalcik 1986; Wredle et al. 2004). It may be that the substantial delay between initial conditioning and retesting was too great for heifers to recall their previous training or that the initial training was not of sufficient duration to leave a lasting impression or recall.

Since neither naïve nor pre-trained heifers urinated more than their yoked controls it is clear training was not effective in building the desired association between the stall and urination. The main differences between urination training in the previous chapter and the current study are the age of animals involved, the frequency of training sessions and the reward. A previous study found heifers learned a spatial discrimination task faster than mature cows, but performed poorly when retested 6 weeks later (Kovalcik & Kovalcik 1986). It may be that while the calves in Chapter 3 readily learned to associate stall entry and urination, this was forgotten in the months between the two studies and the association was more difficult for older animals to acquire. Heifers were noted to be more easily distracted and difficult to handle than calves and this may have contributed to their poor learning performance.

An alternative, or perhaps additional, factor which may have contributed to the heifers' failure to form an association between the stall and urination behaviour is the increased inter-trial interval (the time between trials) which can have a pronounced effect on learning. In the previous chapter calves were trained daily. However the number of animals in the current experiment meant that it was only possible for heifers to be trained every other day, effectively doubling the inter-trial interval and possibly disrupting operant conditioning.

Unlike the previous study with calves, frequency of vocalisation and defecation in the stall did not differ between operant heifers and their yoked controls (t test; $P = 0.98$, $T = 0.02$ and $P = 0.99$, $T > 0.00$). In the previous study yoked control calves vocalised more than the operant calves. Cattle are known to vocalise when stressed (Watts & Stookey 2000). Predictability reduces stress and the lower vocalisations emitted by operant calves may be indicative of lower stress as a result of perceived control over their restraint in the stall. The fact that this was not seen in the current study is further evidence of heifers receiving training failing to understand what is required.

While an association between entering the starting stall and urination was not successfully formed in the current study, this is not to say it is not possible. Further study with greater sample sizes needs to be done to refine the training process and examine factors which can improve cattle's ability to generalise a learned task.

5.2. References

Bittman, S., Mikkelsen, R., 2009. Ammonia Emissions from Agricultural Operations: Livestock. *Better Crops*, 93, pp.28–31.

Collins, F., 1989. Characteristics of Spatial Memory in Cattle. *Applied Animal Behaviour Science*, 23, pp.331–340.

de Passillé, A.M., Rushen, J., Ladewig, J., Petherick, C., 1996. Dairy Calves ' Discrimination of People Based on Previous Handling. *Journal of Animal Science*, 74, pp.969–974.

Gellerman, L.W., 1933. Chance orders of alternating stimuli in visual discrimination experiments. *Journal of Genetic Psychology*, 42, pp.206-208.

Hafez, E.S.E., Schein, M.W., 1962. The behaviour of cattle. In E. S. E. Hazef, ed. *The behaviour of domestic animals*. London: Bailliere Tindall and Cox.

Hultgren, J., Bergsten, C., 2001. Effects of a rubber-slatted flooring system on cleanliness and foot health in tied dairy cows. *Preventive Veterinary Medicine*, 52, pp.89–75.

Kovalcik, K., Kovalcik, M., 1986. Learning ability and memory testing in cattle of different ages. *Applied Animal Behaviour Science*, 15, pp.27–29.

Reneau, J.K. Seykora, A.J., Heins, B.J., Endres, M.I., Farnsworth, R.J., Russell, F.B., 2005. Association between hygiene scores and somatic cell scores in dairy cattle. *Journal of the American Veterinary Medical Association*, 227, pp.1297–1301.

Renken, W.J., Howery, L.D., Ruyle, G.B., Enns, R.M., 2008. Cattle generalise visual cues from the pen to the field to select initial feeding patches. *Applied Animal Behaviour Science*, 109, pp.128–140.

Rybarczyk, P., Rushen, J., de Passillé, A.M., 2003. Recognition of people by dairy calves using colour of clothing. *Applied Animal Behaviour Science*, 81, pp.307–319.

Vaughan, A., de Passillé, A.M., Stookey, J., Rushen, J., 2014. Operant conditioning of urination by calves. *Applied Animal Behaviour Science*, 158, pp.8-15.

Watts, J.M., Stookey, J.M., 2000. Vocal behaviour in cattle : the animal ' s commentary on its biological processes and welfare. *Applied Animal Behaviour Science*, 67, pp.15–33.

Whistance, L.K., 2009. Eliminative behaviour of dairy cows. *Core Organic project Series*, pp.10–22.

Wredle, E., Rushen, J., de Passillé, A.M., Munksgaard, L., 2004. Training cattle to approach a feed source in response to auditory signals. *Canadian Journal of Animal Science*, 84, pp.567–572.

5.3. Conclusions and link to next chapter

Heifers that were successfully trained to urinate in the stall as calves using operant conditioning (Chapter 3) failed to successfully generalise this knowledge to their new location. Contrary to calves in Chapter 3, naïve heifers (n = 5) did not appear to build an association between entering the stall and urination following training. There are many reasons why this may have occurred. Age may have been a factor as heifers have been reported to be more difficult to train due to being easily distracted, particularly around estrus. Alternatively, heifers may simply not have retained their training, 6 months having passed since initial training. Additionally the experimental set up may also have not been sufficiently similar to the one used in Chapter 3 for generalisation to occur.

We used a coloured board around the reward teat during operant conditioning for the calves (Chapter 3). This was used to help calves find the reward teat rapidly but also to provide a distinct cue to use when testing these animals 6 months later. In this chapter the grain reward was provided in a bucket in a stand made of the same coloured board as found around the teat in the previous chapter. It may be that this colour cue was not sufficiently salient for calves or heifers to have noticed. Selecting cues which are specific to certain tasks could help cattle to generalise between locations and across time.

In the following chapter we explore how calves use colour cues to find the location of a milk reward in a Y maze discrimination task. Of particular interest is the hierarchy of cues, for example we know spatial cues are particularly important for cattle and this may lead them to overlook visual cues. We also explored the feasibility of using classical conditioning techniques to improve calves ability to focus on visual cues.

6. Calves attentional bias towards location cues in Y maze discrimination test can be reduced using classical conditioning

This chapter presents two experiments concerned with calves use of colour cues in a Y maze discrimination task. In the first experiment, the location of milk reward was predicted by either colour or both colour and arm of the Y maze. In the second experiment, classical conditioning of colour cues was used to improves calves performance in the Y maze.

Chapter 6 is in preparation for submission for publication.

This manuscript was originally drafted by Alison Vaughan with suggested comments from Drs. Anne Marie de Passillé, Jeffrey Rushen and Joseph Stookey. Experimental design, animal handling, data collection and analysis was under taken by Alison Vaughan with advice from Dr. Jeffrey Rushen and Anne Marie de Passillé.

6.1. Abstract

Cattle perform well in visual discrimination tasks and have been demonstrated to use visual cues to locate food reward . Using visual cues to signpost feed locations may be an effective way of helping cattle adjust to a new environment, for example during weaning, when calves could be encouraged to visit grain feeders. We conducted two experiments to investigate whether prior exposure to a colour cue would aid calves to subsequently select the correct choice in a Y maze using colour cues. In Experiment 1, 21 Holstein calves (four male, seventeen female, 1-14 weeks of age) were either given prior experience of the Y maze where either both side and colour predicted location of reward (n = 11) or colour cues alone were the predictors (n = 10). Calves provided two cues were then retested using only colour cues. In Experiment 2, 19 female Holstein calves (2-6 weeks of age) were either classical conditioned to associate one colour with milk reward or were exposed to colour cues which did not predict presence or absence of reward prior (control calves). The performance of all calves in a Y maze colour discrimination test was then recorded. Calves were given 20 trials a day. As calves did not always complete all trials data was instead rolled over to give sessions of 20 completed trials. Calves were considered to have learnt when they achieved $\geq$16 correct choices out of 20 (Experiment 1: within one session, Experiment 2: within a session for two consecutive sessions). More calves given location and colour cues met the learning criteria than those given colour cues alone. However, location and colour cue calves did not perform better than colour cue calves when subsequently tested with only colour cues ($X^2 = 0.2$, df = 1, P = 0.65). It appears that, in the presence of a more salient cue such as location, colour cues may be overlooked. Nine out of ten classically conditioned calves learnt but none of the control calves met the criteria within the allotted number of sessions. The most common error made by both classically conditioned and control calves was to choose the same side as their previous choice. Five out of seven control calves displayed a clear side preference but this was only seen with one classically conditioned calf. Classical conditioning can be used to increase the speed of learning colour cues and may reduce the development of a side preference. Improving understanding of how cattle learn cues may help us improve training protocols.

Keywords: dairy calves; discrimination; learning; cue; colour

6.2. Introduction

Training plays an important role in the everyday management of cattle at all levels of mechanisation, whether it's a farmer calling his cows for milking or the training of calves to use an automated milk feeder. With the increasing use of automated systems in the agricultural industry today, particularly within dairy farming (Rushen 2008), maximising the usage of these systems to their highest potential is of growing interest. It is important to have an understanding of how specific farm species learn in order to make use of operant and other learning techniques, as well as in establishing animal behaviours more compatible with farming operations (Kilgour 1987). However, whilst the trainability of cattle has been recognised it could be argued that the ability of cattle to learn has not yet been fully utilised, despite suggestions that it be incorporated more in farming practice (Kilgour et al. 1991).

Training also plays an important role in an experimental setting, for example a number of cattle studies have employed operant and classical conditioning protocols as part of their methodology (Uetake & Kudo 1994; Rushen et al. 1998; Wredle et al. 2004; Daros et al. 2014). An important factor in the success of these training protocols is the use of species appropriate cues which are tailored to the species' sensory and cognitive abilities. For example if using visual cues, selected colours must be in the range easily discriminated by the target species and these must be presented at an appropriate height and size. Although colour cues have been used in research to examine visual discrimination, colour cues are not commonly used to control movement of cattle on farm.

Calves have been shown to have the ability to discriminate between two people wearing different coloured clothing but were not able to do this when the same colour clothing was worn (Rybarczyk et al. 2003). In other studies however, cattle have demonstrated an ability to use other characteristics such as body height or facial features to differentiate between individual humans wearing the same colour clothes (Rybarczyk et al. 2001). One explanation for these differences may be that in the presence of a particularly salient cue other cues are overlooked (Simons & Chabris 1999).

The type of errors animals make in discrimination studies is rarely reported. Studies involving training cattle to perform a task generally report binary outcomes (i.e. correct/incorrect). The animal is considered to have learned or failed to learn the required task based on whether the correct response is offered. However, examining the type of errors animals are making could shed light on why animals are making incorrect choices.

Information on why learning criteria are not achieved may inform us of ways to refine training protocols.

This study aims to assess whether colour cues can be learned in the presence of a more salient cue and whether classical conditioning can improve performance in a colour discrimination Y maze test. We also aim to investigate the type of errors most commonly occurring during a simple visual discrimination learning test.

6.3. Materials and Methods

This study was conducted at the University of British Columbia's Dairy Education and Research Centre in Agassiz, BC, Canada. All experimental conditions and procedures were ensured to meet the requirements of the Canadian Council for Animal Care.

6.3.1. General housing and management

All calves were removed from their dam, transferred to an individual concrete floored pen (1.22m x 2.44m) with sawdust bedding and fed 4 L of (high quality) colostrum within 6 hours of birth. Within the first 24 h of age, calves were weighed and identification ear tags attached. While housed individually, female calves were fed 12 L and male calves 8 L of milk per day across two meals (approximately 0800 h and 1500 h). At 6 days of age (±1 day), heifer calves were moved to a group pen, where they were housed in groups of 4-9 animals. Bull calves were moved to a separate group pen on the same day (5-14 days of age). Calves remained in these stable groups for the duration of the experiment.

Group pens measured 7.08m x 4.74m and comprised a slatted area at the front of the pen where the feeders were located and a larger concrete area with sawdust bedding to the rear. Each group pen was equipped with one DeLaval® automated milk feeder CF 1000 CS Combi, one DeLaval® automated grain feeder CF 1000 CS Combi (DeLaval, Tumba, Sweden), as well as one Insentec hay feeder and one Insentec water feeder (Insentec, Marknesse, Holland). Ear tags were fitted with transponders which communicate with feeders to allow feed and water intakes of individual calves to be recorded.

DeLaval feeders measured the daily individual milk and grain intakes for each calf using the volume of feed dispensed. The automated milk feeder provided calves with filtered, pasteurised waste milk via an artificial teat. Data from milk and grain feeders were recorded by Kalbmanager and Win_Institute programs (Foerster-Technik, Engen, Germany). Insentec feeders were equipped with hydraulic scales which allowed for consumption of hay and water to be recorded. Grain feeders dispensed commercial calf starter mix at a maximum rate of 9 kg per day (i.e. *ad libitum*), whilst hay and water were freely available.

Female calves of Experiment 1 had access to 12 L of milk per day (i.e. *ad libitum*) until weaning. For the purposes of another experiment, female calves were assigned to one of three weaning treatments; milk reduced according to grain intake or milk reduced across 5 days, commencing at either 35 d or 77 d. With the exception of 1 calf (#2029) tested during weaning, all heifer calves were tested either pre- (n = 6) or post-weaning (n = 10). Bull calves (n = 4) had access to 6 L of milk per day via the automated feeder and were all tested prior to weaning.

In Experiment 2, heifer calves (n = 19) were all tested prior to weaning. These calves were not part of the previous experiment. The day before beginning training, daily milk allowance from the automated milk feeder was reduced from 12 L to 6 L of milk per day. This was done to increase calves' motivation for milk reward. This was further reduced to 4 L per day prior to beginning the testing day in response to calves increased drinking speed (and thus increased milk intake) during experimental sessions.

Calves remained in group pens for the duration of the experiment and were only removed to take part in training and testing sessions. These sessions took place within the same barn. For training and testing sessions calves were taken individually from their group pen to the experimental pen, where they were visually but not audibly isolated from other calves. None of the calves had taken part in a training experiment prior to this.

6.3.2. Experimental Treatments

6.3.2.1. Experiment 1

This experiment was conducted to test whether prior exposure to a Y maze discrimination task, where both colour cues and side indicated location of milk reward, would improve calves' subsequent performance in a Y maze where colour cues alone indicted location of milk reward.

Calves were trained in a Y maze discrimination task where either side and colour (n = 11) or colour alone (n = 10) predicted location of milk reward (Fig. 6.1.). Once calves trained with both colour and side cues had successfully met the learning criterion (≥16 correct choices out of 20) they began a second phase, in which the side the milk reward was presented on was randomised and colour alone predicted location. Calves were balanced between treatments for weight and age.

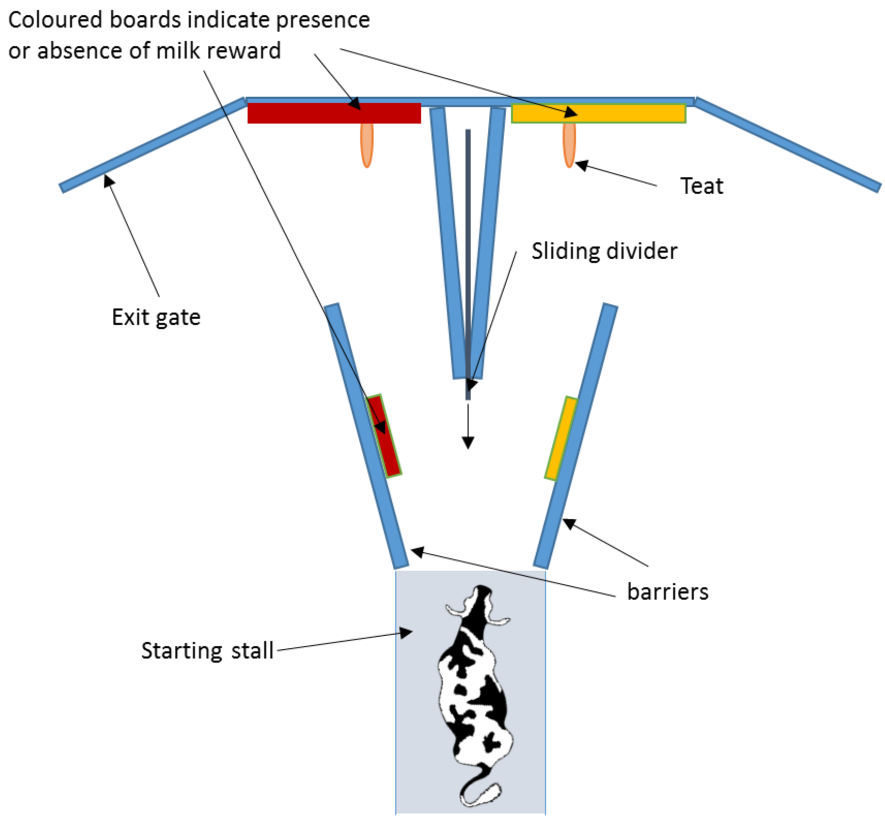


Figure 6.1. - Lay out for Experiment 1

For the two days prior to training, calves were taken once a day from their home pen to the Y maze arena to habituate them to the set up. Colour cues and milk reward were not available, although dummy teats were in place. Calves were held in the starting stall for 20 seconds before the stall gate was opened to allow calves to enter the Y maze. If the calf had not exited the starting stall after 1 minute, pressure was gently applied until the calf entered the Y maze. Calves were allowed 3 minutes to explore the Y maze.

Each experimental day consisted of 20 trials. On the first experimental day the first four trials were "forced choices" where calves were guided into rewarded and unrewarded arms of the Y maze in alternating order. The purpose of this was to familiarise calves with both sides of the Y maze and the cues which predicted the location of the milk reward. During 'forced choices' one arm of the Y maze was blocked and calves were directed into the remaining arm of the Y-maze. The protocol followed an identical procedure to that of the habituation phase with animals being held in the start pen for 20 seconds before being released into the Y-maze. Following the four forced trials, calves were given access to both arms of the Y maze to allow them to make a “free choice” for the remaining 16 trials of the session. For the remaining experimental days, calves were given free choice for all 20 trials within a session.

Once calves had entered one arm of the Y-maze, they were not permitted to move into the other arm. A choice was considered made when the calf’s shoulder had crossed the decision line. If two minutes elapsed without the calf making a choice this marked as an incorrect choice. When entering the rewarded arm of the Y maze calves were able to drink 100ml of milk via a teat (Fig 6.1.). If the calf did not touch the teat within one minute the calf was guided towards the teat by the handler. For an incorrect choice, the teat was removed as soon as the calf’s shoulder crossed the decision line. A sliding divider was placed between the two sides to prevent calves from switching sides and calves were held in the unrewarded arm for 20 seconds. Upon completion of each trial calves were moved back to the starting stall via the exit gate of the chosen arm of the Y-maze.

Calves were considered to have learned the discrimination task when they achieved a criterion of $\geq$ 16 out of 20 correct trials (within one session). Calves not reaching this criterion within 15 days were excluded from the remainder of the experiment.

6.3.2.2. Experiment 2

Experiment 2 was carried out to examine if calves can be classically conditioned to be more attentive to colour cues. Calves (n = 19, 2-6 weeks of age) were assigned according to birth order to either classical conditioned treatment, where a colour was associated to indicate presence or absence of milk reward, or as controls, where they were exposed to colour cues which did not predict presence or absence of reward. During classical conditioning calves were not presented with a choice, rather the teat and colour cues were presented in front of the calf (Fig 6.2.).

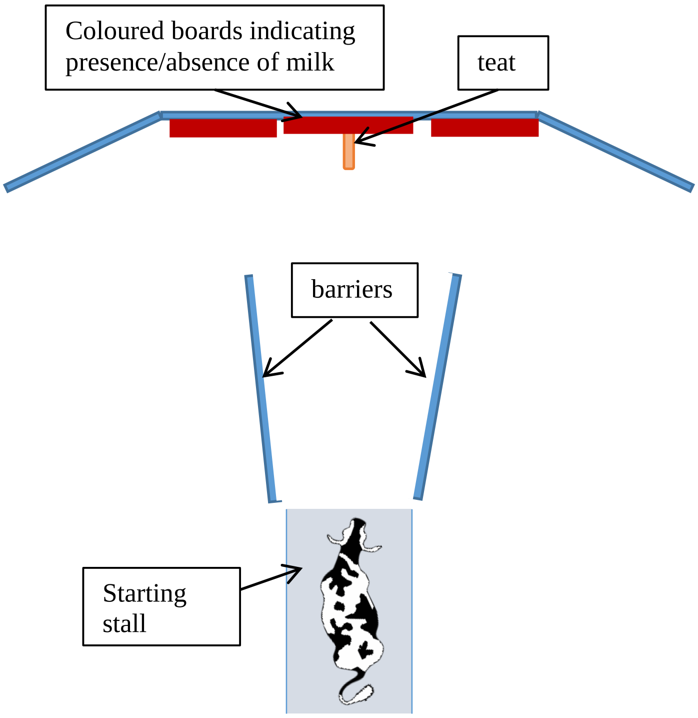


Figure 6.2. – Layout of experimental pen for classical conditioning.

During the conditioning period experimental sessions (20 trials) were divided between the morning and afternoon period, Mon-Fri, beginning at approximately 0800 h and 1500 h.

Trained calves were assigned either red or yellow as their rewarded colour. Initially, conditioned calves were placed individually in a starting stall, facing their rewarded colour and milk reward was available at every trial. Unrewarded trials were gradually introduced with successive sessions until 30% of trials were unrewarded (see Table 6.1.). Control calves were given the same number of reward and unrewarded trials as trained calves but red and yellow colour cues were presented according to a Gellerman series at a ratio of 1:1 (Gellerman 1933). Calves exited the Y maze arena via a side gate on either the left or right according to a different Gellerman series than that used for control calves' colour cues.

Table 6.1. – No of unrewarded trials on each day of classical conditioning, out of a total of 10 trials per session (for both morning and afternoon sessions)

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
No of unrewarded trials (out of 10)	0	0	1	2	3	3	3	3	3	3

On rewarded trials, calves were permitted to drink milk from the teat for 20 seconds (approx. 20 mL, giving a total of 2 L over the 10 trials). On unrewarded trials, the teat was removed if touched and calves were held for 20 seconds before the side gate opened and calves were led back to the start gate for the next trial to commence. If calves did not approach the teat within 20 seconds they were guided to it (if rewarded) or the side gate was opened to allow them to exit (if unrewarded). If calves failed to approach the teat for 4 successive trials the training was discontinued for that day and they were returned to their home pen.

Following completion of the 10 training days calves entered the testing period. During the testing period, calves were brought to a Y maze once a day for testing, beginning at approximately 0900 h. The protocol was similar to that of the training phase; calves were brought individually to the same experimental pen as used in the training phase and the set up was of identical dimensions except a divider was placed to create two sides. Each test day calves could complete up to 20 trials. A trial was considered completed when the calf selected a side of the Y maze within 20 s of leaving the starting stall. Calves did not always complete all 20 test trials within each day. If calves failed to make a choice for 4 successive trials the training was discontinued for that day and they were returned to their home pen. For this reason, a rolling total of completed trials was used for analysis, with 20 completed trials considered a "session". Calves were brought for testing once a day, 5 days a week until calves either met the predetermined criterion of $\geq$16 out of 20 correct trials in two consecutive sessions or completed a minimum of five sessions within 15 days or a maximum of ten sessions. Those calves failing to complete > 5 sessions within 15 days were excluded from the experiment (n = 7, lethargic due to sickness).

Calves were taken individually to the experimental area for testing. Set up was identical to the conditioning step except two teats replaced the central teat and a divider was added between them (Fig. 6.3.).

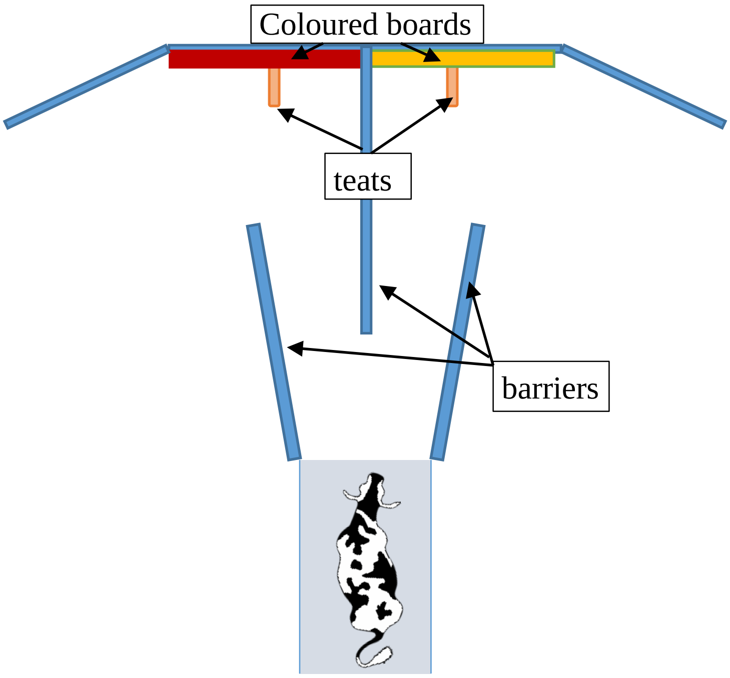


Figure 6.3. – Layout of experimental pen for Y maze testing (Exp. 2).

Control calves were assigned a rewarded colour (as with trained calves in step 1). Location (i.e. left or right) of the rewarded colour cue (and the milk reward) and unrewarded colour cue was alternated according to a Gellermann series. Calves approaching the rewarded colour and touching the teat (a correct choice) in the Y maze were permitted to drink for 10 seconds before returning to the start stall to begin the next trial. If calves approach the unrewarded colour and touched the teat (counted as an incorrect choice), the teat was pulled back and calves given 20 seconds to walk to the other side with the rewarded colour. This was to ensure calves explored both sides of the Y maze. If calves failed to touch either teat within 20 seconds for 4 consecutive trials testing was discontinued for that day and they were returned to their home pen.

Calves were considered to have learned if they achieved ≥ 16 out of 20 correct choices for two consecutive sessions. Calves which did not meet the learning criteria or failed to complete a minimum of 5 full sessions (100 completed choices) within 15 days were not included in analysis.

6.3.3. Statistical analysis

The colour and the arm of the Y maze chosen by calves and whether they were correct was recorded for each trial. In Experiment 2, calves were often observed to switch sides during testing, turning immediately upon entering an arm of the Y maze and entering the other side. If calves did not attempt to touch the teat in the first arm they entered and turned into the second arm without assistance the latter was considered the calves' choice for that trial. Trials where a calf failed to make a choice were removed from analysis. All analyses were calculated per session rather than per day.

The total number of calves meeting the learning criterion (a statistically significant threshold of $\geq$ 16 out of 20 correct choices achieved within one session in Experiment 1, or for two consecutive sessions in Experiment 2) and the number of sessions to achieve this, were calculated. The errors made by calves within each session were recorded and categorised according to whether their previous choice was on the same or opposite side and whether it was correct or incorrect.

Within each experiment we used chi squared analysis to compare the number of calves which met the learning criterion in different treatments. Differences in the number of sessions to achieve the learning criterion was analysed using a student t test. To examine the difference in calves' performance (i.e. number of correct choices) between sessions in Experiment 2, a paired t test was used.

Descriptive statistics were carried out within Microsoft Office Excel (2010) and analysis were conducted using SAS (SAS Institute, 2011).

6.4. Results

6.4.1. Experiment 1

More calves met criterion for learning when both colour and side indicated location of milk reward than those provided with only colour cues (X^2 = 4.3, df = 1, P = 0.03). Although some calves in the single cue treatment achieved the criterion of 16 correct choices out of 20 using colour cues, this took almost 5 times more sessions than those calves which were also given milk always on the same side (mean ±SD: colour and location cues = 2.10 ± 0.32 and colour cues alone = 9.60 ± 3.91; student t test: t=6.26 df= 13 P <0.001).

However, when subsequently tested using colour as the only cue, those calves trained with two cues performed no better at selecting the correct colour than those which had no previous training. Performance was not improved in those calves given previous exposure to colour cues alongside spatial cues; a similar number of calves met the learning criteria (X^2 = 0.2, df

= 1, P = 0.65) and within a similar number of sessions (mean ±SD: retested calves = 9.83 ± 2.79 and naïve calves = 9.60 ± 3.91; student t test: t=0.12 df=9 p=0.91).

6.4.2. Experiment 2

Classically conditioned calves failed to meet the number of correct trials required for the success threshold in the first session (Fig 6.4). Nine out of ten classically conditioned calves met the learning criterion in the Y maze discrimination test within the permitted number of sessions (≥16 correct choices out of 20 trials for two consecutive sessions). In comparison none of the control calves achieved the criteria for learning within the allotted number of testing sessions ($X^2 = 15.39$, df = 1, P < 0.001), although two control calves achieved ≥16 correct choices out of 20 on their last session (Fig 6.4.). One of these calves was not permitted an additional day as she had already reached the maximum number of test days (15 d) while the other calf had reached the maximum number of completed sessions (10 sessions).

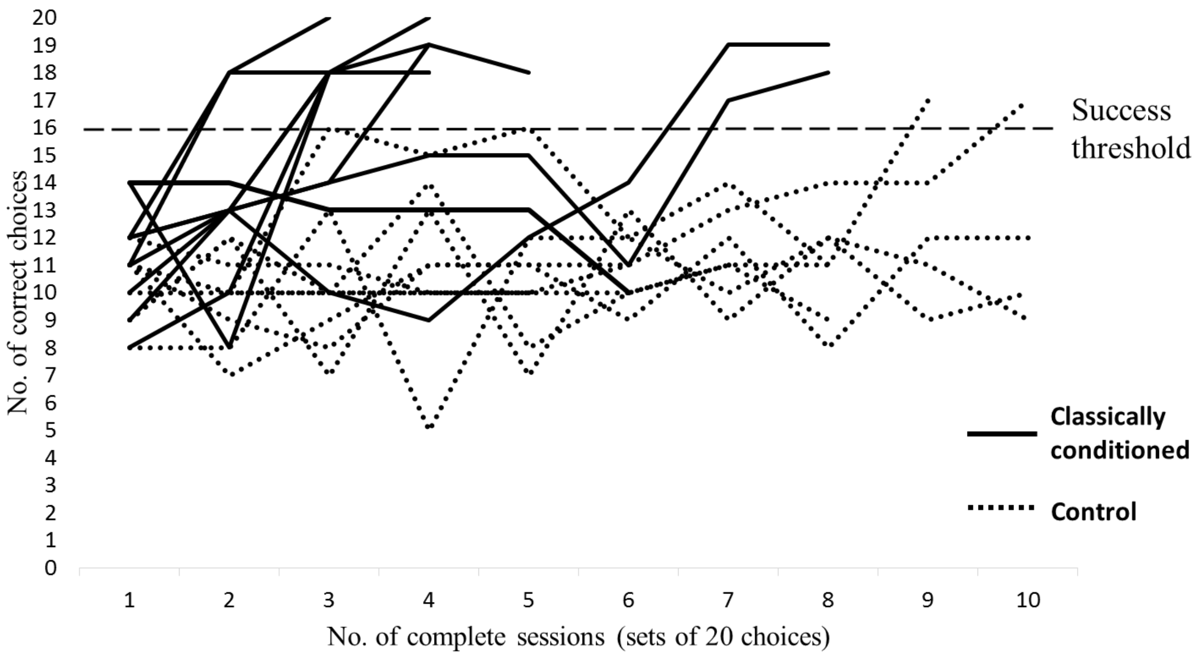


Figure 6.4. - Number of correct choices in each completed set of 20 choices. Solid lines indicate classically conditioned calves (n = 9), dashed indicate controls (n = 9). Criteria for learning was ≥16/20 correct choices for two consecutive sessions.

On average it took classically conditioned calves five sessions to meet the learning criterion (max. number of sessions permitted = 10). Improvement in performance was not gradual

(Table 6.2.). There was a marked increase in the number of correct choices within a session between the final unsuccessful (<16 out of 20) and the first successful session (≥16 out of 20). This was not seen between the previous two unsuccessful sessions (paired t test; T = 4.47, df = 4, p=0.01) nor between the first session and the final unsuccessful session, although only two classical conditioned calves took more than 4 sessions to meet learning criterion so this could not be tested statistically.

Table 6.2. – change in number of correct choices within sessions for calves which met or exceeded the success threshold of 16 out 20 correct choices.

Calf	**last unsuccessful session – 1st session**	**Last unsuccessful session – penultimate unsuccessful trial**	**1st successful session – last unsuccessful session**
3116†	-	-	2
3120	-	2	8
3049	-	4	6
3051	3	0	4
3053	-	1	7
3056	1	3	5
4025†	-	-	4
4052††	3	0	2
4027††	0	-3	6

† achieved learning criterion in < 4 trials

††control calves which reached success threshold

6.4.3. Errors

Classically conditioned calves made less errors per session than controls (mean = 4.1 and 9.4, respectively). The most common error for both control and treatment calves resulted from selecting the same side as the previous trial, although this was more pronounced in control than treatment calves (Fig 6.5.). Only one of the classically conditioned calves did not meet the learning criterion and the errors made by this calf were markedly different, with the calf appearing to switch sides following a correct choice.

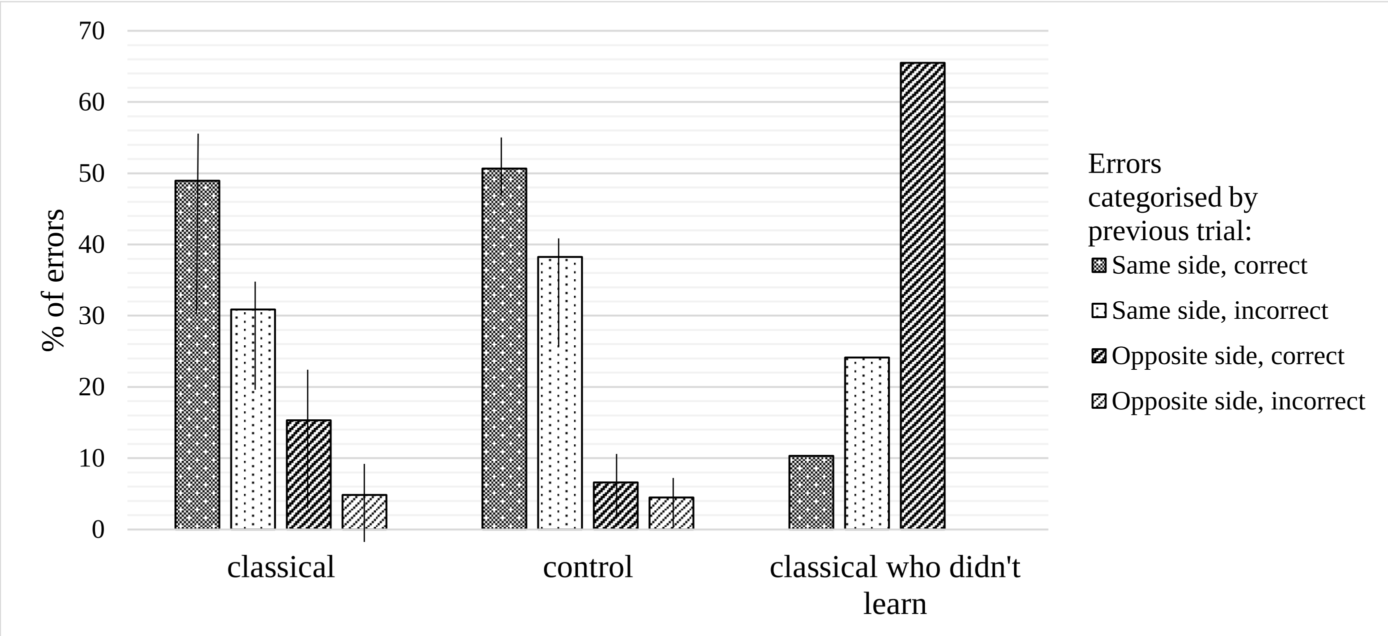


Figure 6.5. - Mean percentage of errors categorised by previous choice; same side and correct, same side and incorrect, opposite side and correct, opposite side and incorrect for classically conditioned calves meeting success criterion (n=7) and control calves (n=7) and the one classically conditioned calf who failed to meet the success criterion.

To test whether these errors were the result of a side preference we examined the frequency which each side was selected in each session. If more than 75% of choices occurred on the same side within a session it was considered a side preference. With the exception of one calf (#3156), the errors made by classical conditioned calves did not appear to be the result of a side preference sustained over two or more consecutive sessions (Fig 6.6. a). In comparison, five out of seven control calves had a side preference which remained relatively constant across five or more sessions (Fig 6.6. b).

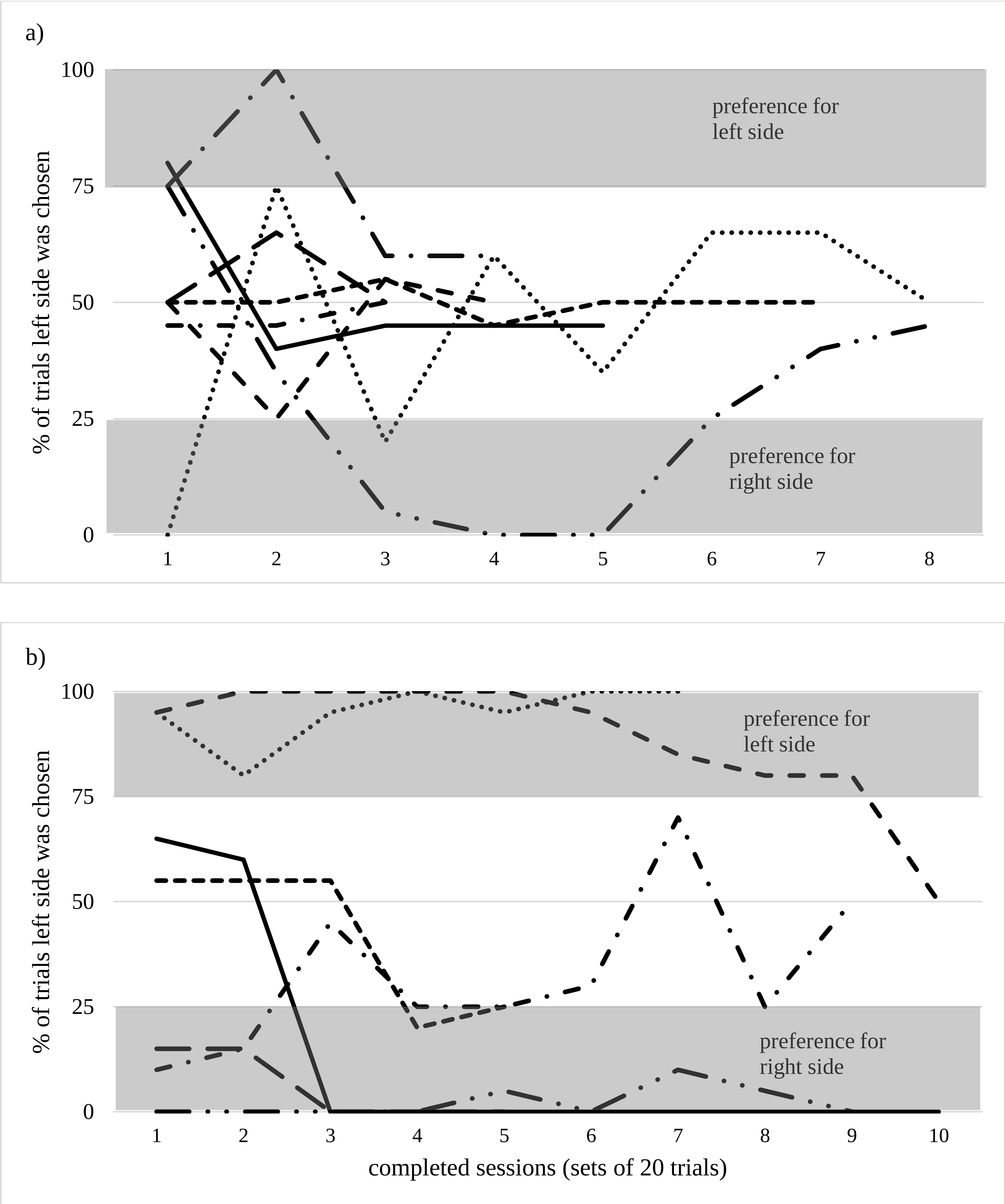


Figure 6.6. - % of trials within each session that classically conditioned calves (a) and control calves (b) chose the left arm of the Y maze. Each line indicates an individual calf. Sessions with ≥ 75% indicate a preference for the left, ≤ 25% a preference for the right.

6.5. Discussion

All calves met the learning criterion when both colour and spatial cues indicated which arm of the Y maze contained the milk reward. In comparison, only half of those calves provided with only colour cues achieved the learning criterion. Additionally those calves presented only colour cues took five times as long to meet the criterion than calves given two cues. It appears as though colour cues may be overlooked when location also predicts milk reward. The majority of calves which underwent classical conditioning (but no control calves) achieved the learning criterion during the Y maze colour discrimination testing. The main error made by both classically conditioned and control calves was to choose the same side as they had chosen in the previous trial, however clear side preferences were only found for control calves.

Calves in Experiment 1 rapidly met the learning criterion when given both location and colour cues but not when given colour cues alone. Providing multiple cues can improve learning of a task, however in this experiment it appears as though calves may have overlooked colour cues altogether rather than using both spatial and colour cues in combination as, when retested with colour cues alone, performance was no better than those calves which had not previously been exposed to colour cues. Similar results were found when cattle were presented simultaneously with both visual and auditory cues, with cattle performing no better than with visual cues alone (Uetake & Kudo 1994).

Cattle rely heavily on spatial cues as they are used daily to navigate within their environment. It is likely that a simple rule such as "always turn right" is easier for cattle to learn than attending to novel colour cues which are unlikely to have had relevance in their everyday life. This explanation is supported by the findings of previous studies looking at the use of visual cues by cattle to discriminate between humans. Rybarczyk and colleagues (2001) found cows are able to make use of many different features of the human appearance to discriminate between two individuals such as height, body recognition (face covered) and facial recognition (both with and without body obscured). In contrast, when the handlers wore different coloured coveralls during training, cattle were subsequently unable to use these other visual characteristics to discriminate between the two handlers (Munksgaard et al. 1999; Rybarczyk et al. 2003). As colour of clothing was likely a very obvious cue it was hypothesised that cows learned to use this at the exclusion of other, more subtle visual discriminators.

Alternatively, the poor performance of calves when switched to colour cues alone may not just result from colour cues being overlooked when provided in conjunction with spatial cues but may also indicate difficulties in changing a learned choice. Grandin and colleagues (1994) reported that cattle resist changing a learned choice in a Y maze test when the rewarded arm was reversed. This effect may be more pronounced in our experiment than in a reversal experiment as the side the reward was presented on was randomised, resulting in calves being intermittently rewarded for choosing their preferred side. Intermittent reinforcement of a learned behaviour actually increases resistance to extinction (Marchant-Forde et al. 2010).

In Experiment 2 we explored whether classical conditioning could be used to make colour cues more relevant to calves. We found that classical conditioning substantially improved both the speed of learning and the number of calves achieving the learning criterion in a Y maze colour discrimination test. All but one of the calves which had undergone classical conditioning achieved the success criterion but none of the controls (although two control calves achieved the success threshold in their final session). On average classically conditioned calves met the learning criteria in half the number of sessions permitted, demonstrating that classical conditioning can be very effective in training cattle to make use of colour cues.

Although calves undergoing classical conditioning outperformed control calves, they did not appear to immediately generalise from the training set up to the Y maze set up. This can be seen by the failure of all calves to exceed the success threshold of 16 out of 20 correct choices in the first session. This could reflect a failure to form an association during classical conditioning but the superior performance of these calves in comparison to controls would seem to discount this explanation. Alternatively it could be that calves required at least one session to become accustomed to the Y maze set up as this was the first time calves were presented with a choice. In other experiments classical conditioned associations between visual cues were successfully generalised to a novel location (Munksgaard et al. 1997; Renken et al. 2008). It may be that generalising to a new location is more readily learned than generalising to a novel context.

Performance did not improve gradually; rather the number of correct choices within a session increased dramatically between the last unsuccessful session and the first successful session. This was seen not only with the classically conditioned calves who learned but also the two control calves who met the success threshold in their final session. This may indicate that these calves would have achieved the success criteria of two consecutive sessions if given an extra training day. Interestingly similar sudden increases in performance reported by Hagen and Broom (2004) were accompanied by measures indicative of increased arousal. This was interpreted as calves exhibiting an emotional response to sudden insights in learning, commonly referred to as the “Aha” experience (Marchant-Forde et al. 2010). However it is difficult to say if this truly represents calves abruptly shifting from no comprehension to full comprehension of the task.

Typically the results of learning experiments are reported in binary terms; animals’ choices are either considered correct or incorrect, with the implicit assumption that incorrect choices are as a result of failure to have understood the task. Calves’ correct choices can only tell us whether or not they have learned the task, however examining the kind of errors calves make during learning may shed some light on the learning process itself.

The most common error made by both classically conditioned and control calves was to choose the same side as they had previously chosen, a pattern which was more pronounced in control calves. Interestingly, the only calf not following this pattern (#4023) was the only classical conditioned calf who did not achieve the learning criteria. These errors were the result of a clear side preference for five out of the seven control calves but a side preference was only shown temporarily for one of the eight classically conditioned calves. This finding agrees with the side preferences found in both Experiment 1 and other studies (Grandin et al. 1994; Paranhos da Costa & Broom 2001), although it suggests that classical conditioning can be used to direct calves attention towards other cues.

Another important consideration when reviewing animals’ performance in learning tasks is to understand that the handler and animal may have a different perception of what is required in the task (Mills 1998). In Experiment 2, calves were not held in the first arm of the Y maze they selected but rather permitted to switch arms. Calves were often highly motivated to enter the Y maze and were observed to rush through the starting stall into the Y maze. Upon entering the unrewarded arm of the Y maze classically conditioned calves were frequently observed to correct themselves; pausing, rapidly turning (without approaching the teat) and entering the correct arm of the Y maze. Rather than making their decision in the starting stall, calves may have only stopped to examine colour cues after entering the Y maze. It may be that increasing the distance between exiting the starting stall and the point at which the Y maze divided would allow calves to make their decision before entering an arm of the Y maze.

Classical conditioning appeared to be more successful than previous exposure alongside a more salient cue in preparing calves to use colour cues to locate the reward in the Y maze set up. However direct comparisons could not be made between Experiments 1 and 2 due to methodological and temporal differences. One of the most important differences between the two experiments, which could potentially have had an effect on the formation and maintenance of side bias, was the consequence of an incorrect choice. In Experiment 1 calves exited from the incorrect arm if chosen. In contrast, calves in Experiment 2 were permitted to switch sides after touching the incorrect teat and only ever exited the Y maze from correct arm. Heifers were not found to develop a side preference in Hagen and Broom's Y maze visual discrimination test (2003), where heifers were permitted to correct their choice if they selected the unrewarded arm. It may be that had this strategy been adopted in Experiment 1, it would have helped calves trained both location and colour to overcome their side bias when tested with colour alone.

6.6. Conclusion

Prior exposure to colour cues alongside spatial cues did little to improve performance in a Y maze discrimination test. However the use of classical conditioning to improve attention to colour cues shows promise. While currently colour cues are rarely used to control movement of cattle, the rapid acquisition of a learned association using classical conditioning may make the use of colour cues an interesting possibility worthy of further attention.

6.7. References

Daros, R.R., Costa, J.H.C., von Keyserlingk, M.A.G., Hötzel, M.J., Weary, D.M., 2014. Separation from the dam causes negative judgement bias in dairy calves. *PloS one*, 9, p.e98429.

Gellerman, L.W., 1933. Chance orders of alternating stimuli in visual discrimination experiments. *Journal of Genetic Psychology*, 42, pp.206-208.

Grandin, T., Odde, K.G., Schutz, D.N., Behrns, L.M., 1994. The reluctance of cattle to change a learned choice may confound preference tests. *Applied Animal Behaviour Science*, 39, pp.21–28.

Hagen, K., Broom, D.M., 2004. Emotional reactions to learning in cattle. *Applied Animal Behaviour Science*, 85, pp.203–213.

Kilgour, R., 1987. Learning and the training of farm animals. *The Veterinary clinics of North America. Food Animal Practice*, 3, pp.269–284.

Kilgour, R., Foster, T.M., Temple, W., Matthews, L.R., Bremner, K.J., 1991. Operant technology applied to solving farm animal problems. An assessment. *Applied Animal Behaviour Science*, 30, pp.141–166.

Marchant-Forde, J.N., McGreevy, P.D., Morton, D.B., Nicol, C.J., Phillips, C.J.C., Sandoe, P., Swaisgood, R.R., 2010. *The encyclopedia of applied animal behaviour and welfare* D. Mills, ed., Wallingford: CABI.

Mills, D.S., 1998. Applying learning theory to the management of the horse: the difference between getting it right and getting it wrong. *Equine Veterinary Journal. Supplement*, 27, pp.44–48.

Munksgaard, L., de Passillé, A.M., Rushen, J., Ladewig, J., 1999. Dairy cows' use of colour cues to discriminate between people. *Applied Animal Behaviour Science*, 65, pp.1–11.

Munksgaard, L., de Passillé, A.M., Rushen, J., Thodberg, K., Jensen, M.B., 1997. Discrimination of People by Dairy Cows Based on Handling. *Journal of Dairy Science*, 80, pp.1106–1112.

Paranhos da Costa M.J., Broom, D., 2001. Consistency of side choice in the milking parlour by Holstein-Friesian cows and its relationship with their reactivity and milk yield. *Applied Animal Behaviour Science*, 70, pp.177–186.

Renken, W.J., Howery, L.D., Ruyle, G.B., Enns, R.M., 2008. Cattle generalise visual cues from the pen to the field to select initial feeding patches. *Applied Animal Behaviour Science*, 109, pp.128–140.

Rushen, J., 2008. Farm animal welfare since the Brambell report. *Applied Animal Behaviour Science*, 113, pp.277–278.

Rushen, J., Munksgaard, L., de Passillé, A.M., Jensen, M.B., Thodberg, K., 1998. Location of handling and dairy cows' responses to people. *Applied Animal Behaviour Science*, 55, pp.259–267.

Rybarczyk, P., Koba, Y., Rushen, J., Tanida, H., de Passillé, A.M., 2001. Can cows discriminate people by their faces? *Applied Animal Behaviour Science*, 74, pp.175–189.

Rybarczyk, P., Rushen, J., de Passillé, A.M., 2003. Recognition of people by dairy calves using colour of clothing. *Applied Animal Behaviour Science*, 81, pp.307–319.

Simons, D.J., Chabris, C.F., 1999. Gorillas in our midst: sustained inattentional blindness for dynamic events. *Perception*, 28, pp.1059–1074.

Uetake, K., Kudo, Y., 1994. Visual dominance over hearing in feed acquisition procedure of cattle. *Applied Animal Behaviour Science,* 42, pp.1–9.

Wredle, E., Rushen, J., de Passillé, A.M., Munksgaard, L., 2004. Training cattle to approach a feed source in response to auditory signals. *Canadian Journal of Animal Science,* 84, pp.567–572.

7. General Discussion, conclusions and future directions

This section does not aim to restate the discussions of previous research chapters but rather to consider those points which have not been comprehensively addressed in the discussion section of each chapter. It also aims to evaluate the importance of the knowledge acquired through the experiments in this thesis.

7.1. General discussion and conclusions

Up until this point in time, the knowledge of calves' urination and defecation habits and our understanding in general of the learning abilities of cattle have been limited. The experiments contained in this thesis were aimed at targeting the most critical points required for latrine training in cattle. Chapter 2 describes, for the first time, the urination and defecation patterns of dairy calves. Chapter 3 served as a "proof of concept experiment' designed to determine whether it is possible for calves to form an association between a location and an elimination behaviour via operant conditioning, lending credibility to the ultimate goal of training cattle to urinate and defecate in specific areas of the barn. Chapter 4 highlights possible age effects on urination training and the failure of previously trained heifers to recall their training, performing no better than naïve heifers when tested again later in a new location. It was hypothesised that one reason for the failure to transfer earlier learning could be that there was not sufficient continuity of visual cues to allow generalisation between the two locations. The colour discrimination experiments described in Chapter 5 demonstrated that classical conditioning could be used to improve calves' focus on colour cues. Within Chapter 5 the errors made by calves during the testing phase of Experiment 2 are also reported. This represents the first time the mistakes made by cattle during the learning process has been examined and offers opportunities for both understanding how cattle learn and improving training techniques.

Chapter 2 represents the first study to report the urination and defecation behaviours of dairy calves. This study of when and where group housed dairy calves urinate and defecate is not only useful in terms of housing design but also in addressing a gap in the scientific knowledge of a fundamental behaviour of calves. For example, the frequency of urination and defecation has been used in various studies as a measure of fear (Van Reenen et al. 2005; De Paula Vieira et al. 2012), with the underlying assumption that emotional response to the experimental set up was the main factor accounting for differences in the frequency of eliminations between individuals. Given the huge daily variation between individuals this assumption needs to be re-examined.

It was not possible using the overhead video camera to reliably discriminate between urination and defecation. In addition, some calves were observed to have brief bouts of diarrhoea which made it difficult to differentiate between urine and diarrhoea in some incidences. Diarrhoea may also influence the daily frequency of urinations and defecations, potentially increasing the frequency of defecation and decreasing the frequency of urination (due to dehydration). However the mean daily frequency of eliminations by calves was correlated between two observation periods (one month apart) which suggests effects of diarrhoea on frequency of total daily eliminations was minimal. The range of total daily eliminations recorded for calves was similar to those reported in adult cattle and, as with adult cattle, substantial variation in the daily frequency of urination and defecation was observed between individuals.

Gaining information on the frequency and variation of daily eliminations was important for designing aspects of the urination training protocol described in Chapter 3 such as optimal time for training sessions (with the activity peaking at 0800 h). Additionally, knowledge of the urination and defecation habits of calves is an important ingredient for the development of a protocol to train cattle to urinate and defecate in specific areas of the barn. Typically training in most species begins at an early age for a few reasons; animals are smaller and easier to control, and typically more receptive to learning. Additionally, in the case of elimination training of cattle, the potential savings in labour and the reduction in the amount of soiled bedding that needs to be replaced, means that it would make economical and practical sense to begin training as early as possible and thereby reap the benefits throughout the animal's life.

Two approaches to building an association between a location and urination were explored in the proof of concept experiments described in Chapter 3. While calves receiving operant conditioning urinated more than controls, classically conditioned calves were no different from controls. This is not to say a classical conditioning approach could not work; training was conducted just once a day for 5 consecutive days and it may be that a learned association could have been achieved had calves been given a greater number of pairings between the location and urination. However some calves trained via operant conditioning required only one or two sessions to acquire the desired response and this seems the most promising approach for future studies to employ.

Other attempts to train cattle to urinate and defecate in a specific location have also used operant conditioning techniques but did not achieve the desired response (Saitoh et al. 2005; Whistance et al. 2009). The operant conditioning experiment described in Chapter 3 is the first time it has been demonstrated that calves can form an association between entering a specific place and urinating to acquire a reward. While the protocols used in this experiment may not be the most practical approach to urination and defecation training on farm, the results of this experiment demonstrate that this is an achievable goal. This experiment also established that calves are capable of making a conscious association between urinating in a specific location and receiving a reward.

Although the operant conditioning training protocol described in Chapter 3 was successful in training one month old heifer calves to urinate in a stall, similar training techniques used with 6 month old heifers failed to achieve a higher frequency of urination within the stall than controls (reported in Chapter 4). While other studies have reported difficulties in training heifers (Entsu et al. 1992), little work has been conducted within the scientific literature to identify the age at which cattle most readily acquire learned associations.

While age differences could be one factor accounting for why training did not increase the frequency of urinations in the stall in 6 month old heifers, it was surprising that those heifers which had successfully learned to urinate upon entering the stall as calves performed no better than naïve heifers. Attempts were made to make the physical set up of the test area for older heifers similar to that used in previous training as calves. However, it is worth contemplating whether the cues which are most easily provided from a management perspective are also the most salient to the animals.

In Chapter 5 it was found that colour cues may be overlooked by calves in the presence of more salient cues. It was found that classical conditioning can be used to rapidly teach calves to use colour cues and reduce the development of side preferences in a Y maze testing scenario. Classical conditioning appeared to help calves to attend to colour cues, which may otherwise be overlooked. Thus such an approach could be used to control cattle's movements using colour cues and to help cattle generalise a learned response to a novel location or context.

7.2. Future directions

There are many factors which need to be addressed before an automated latrine training system could be developed for on farm use. No doubt such a tool would improve the cleanliness of the cattle and barns and help negate the current need for management of manure by means of restrictive housing designs. Chapter 3 marks an important step towards this goal, demonstrating that calves are capable of associating their urination within a specific location with receiving a reward.

While the results of Chapter 3 demonstrate that calves can form an association between a location and urinating to receive a reward, future research could address the assumption that such association could be similarly established with defecation behaviour. Artificial stimulation of defecation is less straightforward than urination which can be rapidly and reliably induced via IV injection of a diuretic, which was a useful tool during operant training. In new-born calves, the dam stimulates both urination and defecation via licking of the urogenital region (Metz & Metz 1986). However it was reported that this reaction is limited to the first day of life. The validity of this claim is doubtful as manual stimulation of the area directly below the vulva is a well-established technique for stimulating urination in adult cattle. It may be possible to induce defecation by manually stimulating the area. However, while it was necessary to induce elimination behaviour for the proof of concept experiment described in Chapter 3, artificial stimulation of urination and defecation may not be essential for training cattle to eliminate in specific areas within the barn. Whistance et al (2009) successfully trained 14-16 month old heifers to associate urinating or defecating with an opportunity to claim a food reward (although their attempts to shape elimination behaviour to a specific location was unsuccessful). While heifer's behaviours following urination and defecation were not reported separately in this paper, the authors claimed that there were no differences in learning speed between defecation and urination.

As was demonstrated in Chapters 3 and 4, future attempts to explore the feasibility of training cattle within their home pen should be targeted at younger animals. Ideally, simple cues could be used to clearly identify target areas, aiding cattle to recognise and generalise their latrine training when moved between pens. While some preliminary studies have been conducted exploring the use of colour cues to control the movement of cattle (Jago & Kerrisk 2011), this is not a commonly used technique. Training cattle to follow visual cues can be a lengthy process. The time required for training may be exacerbated by methodological problems as visual cues being presented in a position which is not optimal for cattle's vision (for further discussion, see Chapter 1). Furthermore if other, more salient, cues are also predictors of reward or punishment cattle may attend to these, overlooking visual cues (Chapter 5).

Motivation is one of the key factors which would determine the success of a system which relies on the cattle to voluntarily move to a specific location. Despite this obvious importance, relatively little is known about what cattle find rewarding, other than feed. Finding viable alternatives to feed rewards would be advantageous as nutrition is critical in dairy cattle management. Cattle's willingness to approach a station for a food reward following an audio cue was dependent on the distance they have to walk (Wredle et al. 2004). To improve cattle's response to the cues it would be necessary either to increase cattle's motivation to access the reward by increasing its value or reduce the distance required to travel to it. Identifying resources which cattle are motivated to gain access to would also have applications beyond urination and defecation training.

Many of the challenges for designing an effective urination and defecation training system, mirror those faced when designing barns for automated milking systems. Barns can be arranged such that moving between feeding and resting areas requires the cow to pass by the milking unit. This layout is referred to as forced or directed traffic. Alternatively, the barn may be set up to allow cows free access to feed, water and a comfortable place to lie down, known as "free traffic" systems. In forced traffic systems cattle spend more time in waiting areas, unable to feed or lie down, which may negatively impact welfare. This is especially pertinent when we consider training of elimination behaviours, as we must consider the substantial variation between individuals in the daily frequency of urination and defecation.

Ideally an automated urination and defecation system would allow for separate collection of urine and faeces. Currently, urine and faeces are collected together from the barn floor and, in many systems, liquid and solid factions are subsequently separated. Immediately separating urine and faeces at the source (i.e. before urine and faeces have had a chance to come into contact) results in reduced ammonia emissions compared with conventional scraper systems (Vaddella et al. 2010). Avoiding mixing of urine and faeces will also reduce the degradation of nutrients, allowing for easier nutrient recovery. Separate collection of urine, in combination with RFID technology, has the potential to automate detection of estrus, pregnancy, ketosis, etc. Thus, the feasibility of separate collection of urine and faeces at targeted areas of the barn which cattle have been trained to visit represents an exciting opportunity for future research.

References

de Paula Vieira, A., de Passillé, A M., Weary, D.M., 2012. Effects of the early social environment on behavioral responses of dairy calves to novel events. *Journal of Dairy Science*, 95, pp.5149–5155.

Entsu, S., Dohi, H., Yamada, A., 1992. Visual acuity of cattle determined by the method of discrimination learning. *Animal Behaviour*, 34, pp.1–10.

Jago, J., Kerrisk, K., 2011. Training methods for introducing cows to a pasture-based automatic milking system. *Applied Animal Behaviour Science*, 131, pp.79–85.

Metz, J., Metz, J.H.M., 1986. Maternal influence on defecation and urination in the newborn calf. *Applied Animal Behaviour Science*, 16, pp.325–333.

Saitoh, T., Seo, T., Kashiwamura, F., 2005. Control of cattle defecation in resting area using feeding station. In *Proceedings of the 39th International Congress of the International Society for Applied Ethology*. Kanagawa, Japan: ISAE2005, p. 127.

Vaddella, V.K., Ndegwa, P.M., Joo, H.S., Ullman, J.L., 2010. Impact of Separating Dairy Cattle Excretions on Ammonia Emissions. *Journal of Environment Quality*, 39, p.1807.

Van Reenen, C.G., O'Connell, N.E., Van der Werf, J.T.N., Korte, S.M., Hopster, H., Jones, R.B., Blokhuis, H.J., 2005. Responses of calves to acute stress: individual consistency and relations between behavioral and physiological measures. *Physiology & Behavior*, 85, pp.557–570.

Whistance, L.K., Sinclair, L.A., Arney, D.R., Phillips, C.J.C., Richard, D., 2009. Trainability of eliminative behaviour in dairy heifers using a secondary reinforcer. *Applied Animal Behaviour Science*, 117, pp.128–136.

Wredle, E., Rushen, J., de Passillé, A.M., Munksgaard, L., 2004. Training cattle to approach a feed source in response to auditory signals. *Canadian Journal of Animal Science*, 84, pp.567–572.