

THE EFFECTS OF PROVIDING SOCIAL AND NUTRITIONAL ENRICHMENT TO DAIRY CALVES  
ON DEVELOPMENT, BEHAVIOR AND LEARNING

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by

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## ABSTRACT

The effects of providing social and nutritional enrichment to dairy calves on  
development, behavior and learning

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The objective of this study was to measure the effects of both a nutritional (water nipple) and social (partner calf) enrichment on calf body weight, grain intake, water intake, behavior and learning. The enrichments included 1) water provided from a nipple vs. a bucket (nutritional) and 2) visual and tactile access to a partner vs. isolated rearing with no visual or tactile access to a partner calf (social). A total of 72 Holstein and Jersey dairy calves were pseudo-randomly distributed into 4 treatments at birth [Individual/Bucket (IB), Paired/Bucket (PB), Individual/Nipple (IN), Paired/Nipple (PN)]. Socially and nutritionally enriched calves drank more water than non-enriched calves (Social:  $5.02 \pm 0.27$  kg/d vs  $3.723 \pm 0.27$  kg/d respectively;  $P = 0.0009$ ; Nutritional:  $4.93 \pm 0.27$  kg/d vs.  $3.81 \pm 0.26$  kg/d respectively;  $P = 0.004$ ). No difference in daily grain intake was found between individual or pair reared calves except during wk 8 ( $1.31 \pm 0.07$  kg/d vs  $1.60 \pm 0.07$  respectively kg/d;  $P = 0.04$ ). There were no differences in average body weight among treatments ( $P > 0.20$ ). Pre-milk delivery, calves reared on a water bucket spent more time standing ( $P = 0.03$ ) and when paired, less time non-nutritively suckling compared to water nipple reared calves ( $P = 0.05$ ). Grooming time was highest during period 2 (wk 3, 4, 5;  $P = 0.01$ ) pre-milk delivery. Post-milk delivery, calves reared on a

water bucket spent more time drinking milk ( $7.13 \pm 0.40$  vs  $5.37 \pm 0.39$  min;  $P = 0.005$ ) and grooming ( $P = 0.05$ ), and less time drinking water ( $P < 0.001$ ) and lying ( $6.17 \pm 1.02$  vs  $9.19 \pm 0.97$  min, respectively;  $P = 0.04$ ) than water nipple reared calves. Water nipple calves when paired exhibited longer drinking times ( $P = 0.04$ ). The most notable behavior was cross suckling post-milk delivery, as the weeks progressed water bucket reared calves increased time spent cross-suckling while water nipple calves maintained the amount of time spent cross-suckling. At wk 8 a subset of 24 calves (6 from each treatment) were trained over 14-d period to differentiate between an “X” and “O” cue to receive a milk reward (visual discrimination task). Learning (% correct choices) was compared using a Wilcoxon-signed rank test. Calves individually reared had greater overall correct choices than pair reared calves ( $0.63 \pm 0.02$  % correct/total choices vs  $0.57 \pm 0.02$  % correct/total choices respectively;  $P = 0.05$ ), while calves reared with a nutritional enrichment (water nipple) had greater overall correct choices compared to water bucket reared calves ( $0.64 \pm 0.02$  % vs  $0.56 \pm 0.02$  %,  $P = 0.02$ ). These results indicate that social and nutritional enrichments positively influence calf cognitive performance, water intake, and lying, cross-sucking, grooming behaviors.

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## CHAPTER 1

### LITERATURE REVIEW

#### 1.1 Dairy Cattle Welfare

Animal welfare is a growing concern among the general public and this has led to the passing of legislation that aims to improve welfare. For example, Proposition 2 was passed in 2008 and went into effect in 2015 establishing new space standards for confinement of veal calves, breeding pigs and egg-laying hens. This piece of legislation was emotionally driven and lacking in empirical evidence to support welfare concerns. Due to the omission of research-based evidence, livestock affected, in particular chickens, faced higher mortality rates when group housed (Mench et al., 2016). While the general concern for animal welfare is present amongst the public, scientists have the responsibility of ensuring there is evidence to support mechanisms behind animal behavior that should ultimately be considered for husbandry changes.

Calf welfare is an important concern in the dairy industry, as calves are the future of the herd. Calves face isolated rearing environments in contrast to the social herd dynamic they will be integrated into later in life. Experience in a stable social group allows calves to form preferred social partners, and their presence can curb stress responses (Reinhardt and Reinhardt, 1981; McLennan, 2012). Calves are immediately separated from the dam to ensure proper health and nutrients are satisfied and then housed individually until weaning where they are then transition into group housing. Two of the

issues with the management system described above are addressed in this thesis: (1) isolated rearing system and (2) the lack of stimulation of natural behaviors.

## 1.2 Conventional Calf Rearing in the US

United States Department of Agriculture's (USDA) 2014 report showed that only 15% of operations raise heifers indoors in multiple-animal groups, while 38% of operations housed heifers individually outside, 7% raised heifers individually inside in heated hutches/pen, and 25% housed heifers individually inside in unheated hutches/pens.

Individual calf rearing systems vary in design, but usually consist of a hutch (1.8 m L x 1 m W x 1.2 H to as large as 2.5 m L x 1.5 m W x 1.5 m H) connected to a fenced outdoor pen (1.8 m L x 1.2 m W x 1 m H). Hutches are typically bedded with a substrate such as straw and furnished with ad libitum fresh water and calf starter offered from buckets.

This is a cost-effective rearing system for the dairy producer and allows for easier individual assessment compared to group housing dairy calves. Calves are housed individually from calving to weaning. Weaning can be as early as 6 weeks of age or when they have started consuming 1.4 kg of calf starter for 3 consecutive days (USDA, 2014).

After weaning calves should stay in their individual hutches for a week to reduce stress (BAMN, 2017) after which they are group housed, experiencing their first novel environment and social hierarchy interactions.

### 1.2.1 Enrichment

Enrichment is defined here as a modification of management or surroundings of the calf that demonstrably improves biological functioning, or other validated measures of welfare over and above what is achieved by following minimum management standards (Mandel et. al., 2016). Incorporation of enrichment yields a stimulating environment that limits hyper-responsiveness to stimuli, particularly present in isolated calves reared in crates (Wilt, 1985) and improves the cognitive development in dairy calves (Gaillard et al., 2014). Enrichment has both long- and short-term effects, contributes differently to each production stage, and can be limited to a brief time window (Mandel et. al., 2016). Enrichment can be broken further broken down into specific subcategories, focusing on particular aspects of its application. For the purpose of this thesis we will only consider social and nutritional enrichment applications.

Enrichment, in the context of dairy calves, is a way of providing resources to exhibit natural behaviors and improve cognitive development- particularly in fixed living environments- social companionship and nutritional enrichment has been studied most to date. Animals with enriched social environments tend to show habituation and less reactivity to novel objects, and a faster rate of learning the rewarded cue with fewer deficits in visual discrimination reversal tests (subjects are tasked with visually discriminating between two cues, once the subject has learned the cue the rules are reversed and the subject is now tasked with learning the other cue as opposed to the

first cue) than animals raised in a non-enriched individual rearing environment (Gaillard et al., 2014; Hovarth et al., 2016).

## 1.2.2 Social and Nutritional Enrichment

### Suckling Behavior

In the U.S., 63% of operations feed milk by nipple as opposed to a bucket or other method of delivery and 95% feed milk twice a day (USDA 2014). Feeding milk this way limits the calf's ability to suckle to two bouts a day for a limited duration depending on the quantity in the bottle. Calves raised with dams nurse, on average, 7.2 minutes with a range from 2.8-16.3 minutes nursing the dam where the suckling behavior takes up 60-70% of the nursing time bout and nurse approximately 8 to 12 times a day on the dam (Reinhardt and Reinhardt, 1981; Day et al., 1987; Lidfors, 1996; Lidfors et. al., 2010).

One aspect of the innate behavior is allowing the calf to suckle continuously until satiated/to completion, which is less likely to occur in artificial rearing systems where calves are fed limited quantities of milk for short periods of time. Physiologically, suckling elicits the release of hormones involved in digestion (de Passillé, 2001; Lupoli et al., 2001) reducing the events of scours and cross/non-nutritive suckling. Cross suckling is more likely to occur in calves that are reared with milk delivery from a bucket instead of a teat (Hammell et al., 1988) indicating the importance of the presence of a nipple.

An increase in non-nutritive and cross-suckling could also be explained as a manifestation from frustration or boredom the calf experiences. Integration of a

nutritional enrichment in the form of a nipple could improve health and welfare by allowing the innate natural behavior of nursing in an artificial rearing environment.

### Early development

The ability for a calf to adapt its behavior is believed to be dependent on early exposure to social and environmental variation (Sackett, 1970) which is critical to the dairy calf's success as they integrate into the herd. Individual rearing established weaker bonds with other calves than calves that were raised in groups, likely because they lacked exposure to social encounters where calves learn to compete for resources and navigate hierarchical interactions (Duve and Jensen, 2011). Research has also indicated that calves housed in pairs or groups from birth experience a positive affective state, often exhibiting play behavior, which occurs when all primary needs are met and may be reinforcing of positive emotions to the calf (Duve et al., 2012; Held and Špinka, 2011). Individually raised calves compared to group calves also startle more, are more fearful, and have an increased heart rate and adrenocortical responses to novel stimuli like restraint (de Paula Vieira, et. al., 2012; Duve et al., 2012; Mandel et. al., 2016; Van Reenen et al., 2005). Positive behaviors like playing (Boissy et al., 2007), bonding (Duve and Jensen, 2011), and reduced fearfulness around farm staff (Lürzel et al., 2015) are products of welfare that targets early development and incorporates enrichment.



## Regrouping

Social enrichment is particularly relevant in early development as it sets a foundation for successful behaviors when regrouping occurs. Integration into the herd for an individually reared calf, because of social deficits, can result in a decreased success in its ability to compete at the feed bunk and are likely more submissive in social encounters (Broom and Leaver, 1978). After regrouping, paired housed calves compared to individually housed calves over a 15d period have a shorter latency to visit the feed bunk ( $9.1 \pm 2.6$  vs.  $49.5 \pm 4.1$  h/calf), increased consumption of concentrate ( $3.4$  vs.  $2.3 \pm 0.2$  kg/d per calf) and higher body weight gains ( $0.5$  vs.  $-2.4 \pm 0.3$  kg/d per calf; de Paula Vieira et al., 2010). Location and selection of feed seems to be more dependent on social learning, where the primary function is to expand the feeding repertoire to include unfamiliar feeds that conspecifics are eating, a situation only available to pair reared calves (Galef, 1977; Galef, 1993). Calves reared in small groups spent less time at the back of the pen, which is where they first entered, were more likely to be at the front of the pen, where the feed bunk was located, and tended to have a shorter latency to begin feeding (Horvath & Miller-Cushon, 2018). Regrouping occurs regularly in the dairy cows' life, and face stressors like increased displacement at the feeding bunk and aggressive feeding behavior (Brakel and Leis, 1976; Keyserlingk et al., 2008); by offering a socially enriched environment during early development calves are more competitive at the feed bunk for these future regroupings.

## Abnormal Oral Behavior

Abnormal behavior can result from the lack of an enriched environment. Individual calves tend to develop self-directed abnormal oral behaviors like cross- and non-nutritive suckling (Veisser et al., 1997). Cross- and non-nutritive sucking is also associated with unwanted issues like navel abscesses and drinking of urine (Veissier et al., 2013) when calves are regrouped. Abnormal oral behaviors have also been shown to be a redirection of natural sucking behavior potentially from the lack of access to a nipple/udder during milk delivery, and the decreased availability of suckling opportunities or the motivation to suckle (Margerison et. al., 2003; Jensen and Weary, 2013). Milk flow rate also affects abnormal oral behaviors; fast milk flow rates decrease the suckling time available for bottle fed calves. Which increases abnormal suckling behavior post milk delivery (de Passille, 2001; Hammell et al., 1988). Adding a slow milk flow nipple is a form of nutritional enrichment that reduces unwanted oral behaviors by satisfying the suckling behavior during milk delivery.

## Cognition

Paired housing also improves cognitive development in calves as opposed to individual housing. When implementing a reversal learning test—a two-phase method used to measure cognition by (phase 1) training positive association to one cue over a second cue via reward until learned and then (phase 2) reversing the cues to test how long it takes the subject to learn they have switched—socially enriched calves learned the reversal learning task in 13 sessions while individually housed calves took 19.5 sessions

to meet the learning criterion (Gaillard et. al., 2014). In a similar study, levels of time spent in social enrichment (individual, paired at 6w and paired at 6d) was found to have poor, intermediate and high success in a reversal learning task respectively (Meagher et al., 2015) suggesting that the length of social enrichment has an increasing positive effect on cognition. This work suggests that failing to provide complex social environments may limit a calf's inability to habituate effectively with weaning, novelty and even social interactions.

### 1.3 Cognition and Learning

#### 1.3.1 Learning in dairy calves

Dairy calves will face novel environments throughout their development, including transitions in housing environments to experiencing the milking parlor for the first time. The ability for calves to learn and adapt to novel situations allows them to become more successful and competitive in future novel environments. The reaction a calf may have to one stimulus is not static, it varies depending on previous learned experiences and memories. Learning can be defined as the acquisition of knowledge or skills through experience, study, or by being taught. Cognition—an integration, processing and response to external and internal stimuli—facilitates learning.

Learning methods and rates vary among individuals, which can be partly attributed to environment and the success rate of receiving a reward. Environment has been shown to have a strong effect on affect state and learning, particularly looking at enrichment.

In a T-maze test calves reared with a social partner completed the task faster and spent less time on the incorrect side than individually reared calves (Horvath et al., 2016). During a reversal learning test calves that were socially enriched began to respond to the rewarded option while individually reared calves continued to perform below chance (50%), indicating socially enriched calves were able to change their behaviors and adapt to new situations (Gaillard et al., 2014).

#### Learning in enriched environments

Past studies have identified a positive correlation between an enriched environment and health and learning success in rats, sheep, and pygmy marmosets for example (Burman et al., 2008; Destrez et al., 2014; Munger et al., 2017). Calf studies where the environment was enriched socially with a partner or group-housing resulted in a decrease in vocalizations in novel object tests and decreased heart rate as well as a stronger learning curve over socially deprived calves (Gaillard et al., 2014), indicating socially enriched calves were less prone to exhibit stress in new environments.

Nutritional enrichment has also been identified as having a positive effect on affect state (emotional state) and learning, measured via cognitive and judgement bias tests. It is hypothesized that the ability to perform natural feeding behaviors allows for cognitive development (Horvath, 2016). This is crucial to the development of a young animal as much of what they experience and explore shape the expression of personality.

Facilitated learning is one aspect of shaping and developing in young animals as they watch conspecifics and older relatives navigate stimulus and their outcomes; this allows

the young animal to experience novel situations and learn the appropriate response visually before attempting itself- often helping to reduce negative experiences and stress/fear reactions.

### 1.3.2 Cognition in dairy calves

Cognition is the mechanism by which animals acquire, process, store and act on information from the environment and encompasses capacities of judgment and learning (Destrez et al. 2013). In animals, particularly domesticated animals, vision is an important component of how they perceive their environment, food sources, social companions and threats. Visual cognition begins with the visual system, as images are processed by the eye they are selected and attended to, and then perceived before moving into memory. How cows cognitively process stimuli is important to understand; we need a grasp of the inner mechanism to appreciate the process of learning from the cow's point of view.

#### Attention

In the case of the animal, attention could best be described as the mechanism that selects particular internal representations of the environment to guide action (Wright, 1998). For example, a cow may survey a field for potential feed sources and avoid areas of sparse vegetation to attend her grazing movement towards dense vegetation. This is the first step in the cognitive process—attending to the object/environment in question by choosing and concentrating on relevant stimuli. The cow is attending to only grazing

the dense vegetation, she is not concentrating on finding water or social companions. Using the example of foraging, once the cow gets to the dense vegetation area, she needs to identify her preferred feed (grasses) which are mixed unpredictably among weeds and landscaping features (Shettleworth, 2010) requiring her to be able to attend to multiple foraging stimuli at once. Attending to many things at once increases the time spent finding a particular forage item (in the cow's case grass specifically) but will improve with time and experience in selecting for the particular forage item as she incorporates the next step in cognitive processes: perception.

### Perception

Visual perception refers to the brain's ability to process and make sense of what is being seen. Invariant properties [unique/defining characteristics] of these objects/environments are assessed by perceptual systems [sight, sound, smell, touch] to guide necessary action (Gibson, 1979). In our example, after the cow has attended to her foraging environment and moved towards the dense vegetation area, she then assesses the types of vegetation in the dense areas to graze upon. This assessment is perception: the cow is using her senses (particularly vision and taste) to identify the grasses from weeds and rocks. The need to perceive visual stimuli is anchored in the evolution of sighted animals, as a main modality for executing behaviors (like the consumption of grass). Perceptual analysis of many objects can be achieved in parallel (meaning the cow can perceive multiple types/species of forage at once, not just one at a time) when the visual stimuli is a familiar or practiced environment. Multiple objects,

and occasionally the entire scene of familiar objects can be represented very quickly, independent of the focus of attention (Houghton & Tripper, 1994) in environments that are familiar; which is why novel environments elicit stress behaviors as the animal has to attend and perceive a multitude of new stimuli at once. Once perception has been achieved the final step in the cognitive process is recognition of the object/environment to act upon that information.

### Recognition

Pattern recognition occurs when information from the environment is received and entered into short-term memory, causing automatic activation of a specific content in long-term memory. Recognizing patterns allow animals to predict and expect what is coming. The process of pattern recognition involves matching the information received with the information already stored in the brain. Making the connection between memories and information perceived is a step of pattern recognition called identification. Pattern recognition requires repetition of experience. For animals, spatial positioning in the environment, remembering findings, and detecting hazards and resources to increase chances of survival are examples of the application of pattern recognition for humans and animals (Mattson, 2014). For our dairy cow foraging among the dense grasses, she has already perceived the grasses (their size, shape, color etc) and now matches those characteristics to the grasses she has stored in her long-term memory. When she gets a match, she recognizes the grass as one she likes and acts accordingly- by eating it!

## Visual Discrimination Cognition

Cows are a diurnal animal, and while they rely on all 5 sensory modalities, vision is the dominant sense (Adamczyk et al., 2015). As prey animals, their eyes are located on the periphery of their heads, allowing for at least a wide field of view of 330 degrees. Cow eye sight is dichromatic, meaning they can distinguish readily between long (red, orange and yellow) and short wavelengths (blue, green). It has been demonstrated that cows pay closer attention to moving objects than stationary ones and are easily frightened by sudden movements which are perceived to have a damaging effect (Bower, 1971; Bower, Broughton, & Moore, 1970).

Visual discrimination is the ability to recognize specific aspects of a visual image that make it unique when compared to others. This task includes the use of perceiving changes in form, shapes, colors and positions of objects, people and printed materials. It involves spatial awareness, memory and cognition in order for the subject to correctly process visual images, and further discriminate between them.

Visual Discrimination behavior is based on individual experience. In the wild, animals must be able to learn simple discriminations, whether when foraging or determining predator/prey. In the lab animals are taught to discriminate between visual cues using operant conditioning. These lab tests are used to determine the effects of changing environments, stress, social factors and other independent variables in the animals' life.



Visual discrimination tests can show the animal's ability to problem solve and adapt behaviors to new stimulus. Calves have been shown to discriminate visually between objects of differing brightness successfully for a food reward (Schaeffer & Sikes, 1970). Bulls are capable of visually discriminating between varying sizes of disks (Rehkämper & Görlach, 1997). In more advanced visual discrimination trials where calves underwent a reversal learning process (when the wrong cue become the correct cue and vice versa), it was suggested that calves that learned the reversal test may adapt quicker to novel environments (Gaillard et al., 2014).

#### 1.4 Study Objective and Hypothesis

Welfare is a growing concern among the public and industry; identifying issues in husbandry to allow for raising both physically and mentally healthy animals is a priority. The lack of environmental stimuli and its consequential effects on calf affect state can be determined during cognitive tests. Social and nutritional enrichment may be tools we can implement to help facilitate cognitive development and learning in the early developmental stages of calf life.

The objective of this study was to determine the effects of two forms of enrichment (nutritional and social) on dairy calf behavior and cognition. A nipple waterer enrichment was employed to allow a nutritive option to perform the suckling behavior, while access to a partner calf was the second form of enrichment to allow for social enrichment.

We hypothesized that both nutritional and social enrichment during the early stage of calf development would improve cognitive and learning abilities. We predicted the combination of both enrichments together would yield better learning effects than the enrichments implemented separately. It is also predicted that the nutritional form of enrichment will increase water intake and the social form of enrichment will increase grain intake at the time of weaning.

## CHAPTER 2

### METHODOLOGY AND PROCEDURES

#### 2.1 Animals, Housing and Diet

This study was conducted with 72 dairy calves (36 Holstein, 36 Jersey; 5 males, 67 females) at the California Polytechnic State University, San Luis Obispo, Dairy Unit.

Calves were housed outside in single fenced hutches. All calf hutches were covered, 1.2 x 0.9 m feet in dimension and bedded with straw; access to an outdoor, 0.9 x 1.2 m fenced area was also provided. Calves had ad libitum access to fresh grain and water. Pasteurized whole milk (5 L/feeding; 10L/d) was fed via bottle with a nipple at 05:00h and 17:00h. Starter calf medicated grain (Cal Poly Feed, Cal Poly Feed Mill) and water were offered ad libitum. Calves were weaned via a two-step program, the first step down occurred at wk 6 (10L/d to 5L/d) and were fully weaned at wk 8 (5L/d to 0L/d).

#### 2.2 Experimental Design and Treatments

Calves were separated from the dam within 12 h after calving, housed in a group pen adjacent to the maternity pen (3.05 x 2.43 m) for 1 to 7d, and then enrolled into one of four treatments: (1) individual housing with water bucket (IB), (2) individual housing with water nipple (IN), (3) paired housing with water bucket (PB), and (4) paired housing with water nipple (PN). The study took place from Jan 2018 to March 2019. Calves were a minimum of 1d to a maximum of 7d of age at enrollment and 63 d (9 wk) of age at disenrollment. Assignment into each treatment was pseudorandom; treatments were

balanced by sex, breed, and date of birth. Pictures of each of the four treatments are provided in Figure 1. Nipples were mounted on a board and secured at head height. A 0.91 m long, 4.8 mm clear vinyl tubing, connected the nipple to a 19 L lidded water bucket. Nipple water flow was checked twice daily and cleaned weekly. Calves not on the nipple treatment received their water in the standard buckets used as part of the farms' standard protocol. Paired calves were housed in two individual pens that were placed next to each other and secured together with zip ties to allow for tactile contact between the pairs. Individual calves were housed in individual pens and had no tactile and limited visual contact (when eating or drinking). Only one calf—the focal calf—of the pair was monitored throughout the study in order to avoid confounding effects in the paired treatments. Focal calves in the pair treatment were enrolled within a week of their partner calves' birth and generally the younger of the pair.



Figure 1. Treatment setup of individually reared calves with a water nipple enrichment (A), individually reared calves with a water bucket (B), pair reared calves with a water nipple enrichment (C) and pair reared calves with a water bucket (D).

A subset of 6 female calves from each treatment were pseudo-randomly selected and balanced by breed for a visual discrimination test during week 8 and 9. The visual discrimination test consisted of two cues (“X” and “O”) that were black on white square boards. Calves were assigned to either cue pseudo-randomly, balancing cues by treatment. Calves were tested at 17:00 h once a day for up to 14 d or until the task was learned. Learning was achieved when calves had three consecutive days of 80% or more correct choices. Each session consisted of 12 trials that did not exceed 2 minutes each in

length. The P.M. milk allotment (5 L) was used as a reward, maintaining standard nutrient requirements. Cues randomly alternated sides, with no more than 6 times per side to ensure the calves learned to visually discriminate between them rather than the location.

Calves were led to the test arena on d 1 of week 8 and allowed to habituate to the arena for 10 min, after which the calf received milk on both sides of the arena with no cues present. There were no forced correct choices administered. Following the habituation period and milk exposure on each side of the arena, the calf began the test.

Latency to select a cue was recorded when the calf's front two legs cross the threshold of the alley way into the test arena and ended when the calf's muzzle touched the back wall of the arena (by the mounted cues) on either side of the middle partition. A calf that did not select a cue within 2 min (maximum time per trial) was considered a failed trial attempt and was returned to the start box by the handler where it was held for 20 s. Calves that selected the correct cue were rewarded with 15 s of milk delivered from a nipple passed through a hole under the correct cue. Testing continued until learning was achieved at an 80% threshold for 3 consecutive days (approached the correct cue 80% of the time) or 14 days had elapsed.

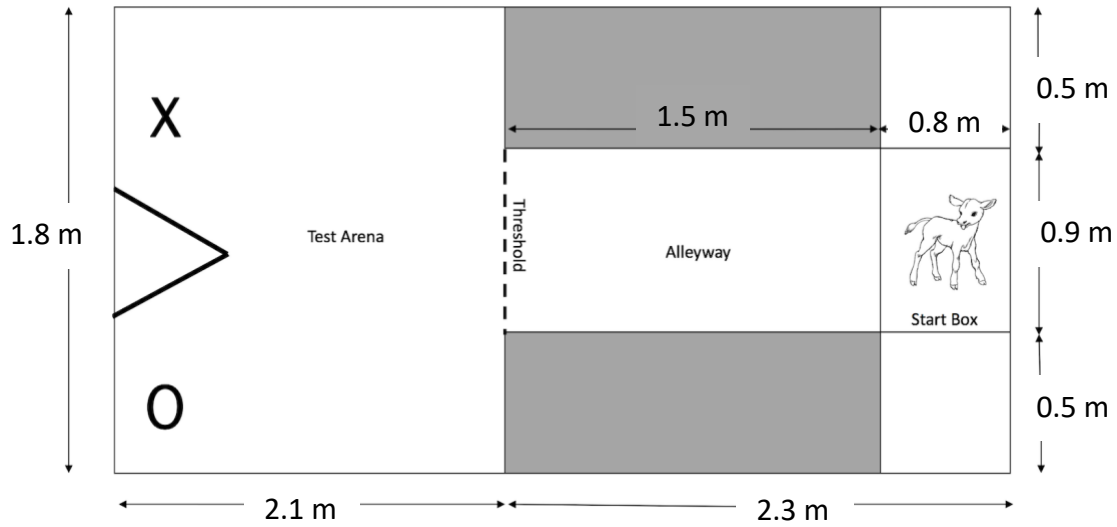


Figure 2. Test arena diagram. A plywood arena (1.8 m X 4.4 m X 1.2 m, Width x Length x Height) was built to house calves during the visual discrimination test. An entrance leading into a 1.5 m long alley way opened to a rectangular space (1.8 m X 2.1 m X 1.2 m, Width x Length x Height) where the two cues (“X” or “O”) were mounted on the back wall.

### 2.3 Data Collection

Data collection occurred over a 9 wk period for all focal calves (Figure 3). Grain intake, water intake, body weight and behavior before and after the P.M. milk delivery, were measured during the first 7 weeks conducted from enrollment to wk7. Test for the visual discrimination task began at wk 8 and concluded at the end of wk 9 when calves were disenrolled from the study.

At 09:00 h grain and water were replenished as necessary to ensure ad libitum availability. Intakes were assessed daily at 15:00 h where old grain and water were discarded and replaced with fresh grain (0.35 - 4.0 kg, dependent on age) and water (18 kg). Grain and water intake were measured in kgs using OHAUS SD35 Bench Scale (OHAUS Corp, NJ). Grain and water weights were recorded when delivered and then before refilling the next day to determine total grain and water intake. Data was not collected when it rained as values were inaccurate due to moisture. An investigation into the evaporation from water buckets for IB and PB treatments did not show differences; there was no concern that evaporation affected water intake values for IB and PB treatments.

Body weight was measured weekly using the Tru-Test EziWeigh5i System along with the Tru-Test Alleyway Platform AP600 (Demanters Limited, Texas, USA).

Cameras (APEMAN Trail camera 12MP 1080P D game & Hunting Camera, US) were used to assess all behaviors (Table 1). Cameras were mounted on wooden towers placed in front of each experimental hutch. Three days of behavioral recordings occurred weekly between 17:00h and 21:00h; cameras were set to take pictures with a time sampling interval of 15 s. For calves in the pair treatment only the focal calf's behaviors were recorded. Behaviors were only considered if the head of the focal calf was in view; when the head was not visible then behavior was recorded as "out of view". For drinking milk,



cross and non-nutritive suckling in addition to the head being in view the muzzle also was required to be in view.

Behavior	Definition
Cross Suckling	Focal calf has made contact with the paired calf and is suckling a body part of partner calf or touching muzzles through the fence
Drinking Milk	Calf has mouth/muzzle over milk nipple
Drinking Water	Focal calf has muzzle inside water bucket (ears past fence) or muzzle over water nipple
Eating Grain	Focal calf has muzzle inside grain bucket (ears past fence)
Lying	Calf is recumbent on 2 or all legs (calf is either in the process of lying down or is lying down)
Non-nutritive Suckling	Focal Calf is seen with mouth/muzzle or tongue on any fixture in pen (except for the water nipple in IN and PN)
Standing	Calf is erect on 4 legs

Table 1. Ethogram of behaviors observed using trail cameras during the 30 min before and the 30 after the afternoon milk delivery.

## 2.4 Statistical Analysis

### 2.4.1 Grain and Water Intake

For each calf, weekly grain and water intake were summarized before determining an average weekly intake by treatment. The effect of each treatment level was tested using the MIXED procedure in SAS (version 9.4). Fixed effects included week, breed, nutritional enrichment, social enrichment and the social x week, nutritional x week and social x nutritional enrichment interactions; week was included as a repeated measure and calf as a random effect.

### 2.4.2 Body Weight

The effect of each treatment level on weekly body weight was tested using the MIXED procedure in SAS (version 9.4). Fixed effects included week, breed, nutritional enrichment, social enrichment and the social x week, nutritional x week and social x nutritional enrichment interactions; with week as a repeated measure and calf as subject

### 2.4.3 Behaviors

Weekly behavior occurrence by calf was calculated by averaging weekly behavior occurrence by the four treatment levels (IB, IN, PB, PN). Each behavior was analyzed separately by the 30 min period pre-milk delivery and 30 min post-milk delivery.

Behaviors were then averaged into three-week periods (period 1: wk 1, 2; period 2: wk 3, 4, 5; and period 3: 6, 7). The effect of each enrichment level was tested using the

MIXED procedure in SAS (version 9.4). Fixed effects included period, breed, nutritional enrichment, social enrichment and the social x period, nutritional x period and social enrichment x nutritional enrichment interactions; with period as a repeated measure and calf as subject.

#### 2.4.4 Visual Discrimination Test

The percent correct choices per calf was calculated by dividing the total number of correct choices by the total number of choices per session (i.e. 12). The number of sessions to reach learning criterion (80% correct choices for 3 consecutive days) was calculated. The effect of each treatment level was tested using the MIXED procedure in SAS (version 9.4). Fixed effects included session, nutritional enrichment, social enrichment, and the social x session, nutritional x session and social x nutritional enrichment interactions; session was included as a repeated measure and calf as a random effect. Time to reach learning criterion was analyzed with a Wilcoxon signed-rank test. There was not strong enough power to include breed in the model.

## CHAPTER 3

### RESULTS AND DISCUSSION

#### 3.1 Water and Grain Intake

##### 3.1.1. Water intake

No difference was found in water intake across the 9 wk sampling period between breed ( $P = 0.75$ ). Water intake increased for all calves over time ( $P < 0.001$ ). Paired calves drank more than individuals overall ( $5.02 \pm 0.27$  vs.  $3.72 \pm 0.27$  kg/d;  $P < 0.001$ ) while calves provided water from a nipple drank more than calves provided water from a bucket ( $4.93 \pm 0.27$  kg/d vs.  $3.81 \pm 0.27$  kg/d;  $P = 0.004$ ). A social enrichment by nutritional enrichment interaction was detected ( $P < 0.001$ ; Figure 3); amongst calves housed individually there was little difference in water intake between those fed via nipple or bucket ( $3.46 \pm 0.38$  vs.  $3.97 \pm 0.38$  kg/d, respectively), however calves housed in pairs with the nipple enrichment drank more than the pairs with buckets ( $6.39 \pm 0.39$  vs.  $3.65 \pm 0.38$  kg/d, respectively). The lack of interaction between week and social enrichment ( $P = 0.18$ ) indicated that pair reared calves drank more water than individually housed calves throughout the 9 wk period (Figure 4a). There was a week by nutritional enrichment interaction ( $P = 0.001$ ; Figure 4b) where calves fed water from a nipple consumed more water than calves fed water from a bucket until week 6 after which there was no difference.

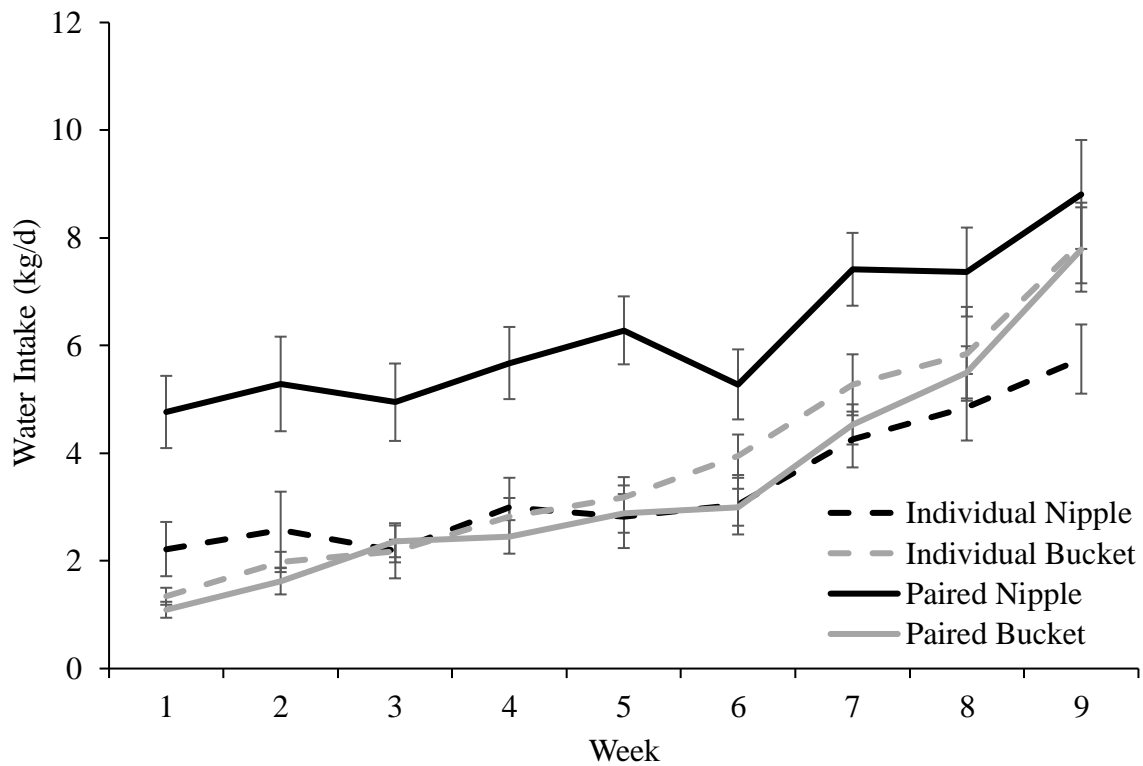


Figure 3. Mean ( $\pm$ SEM) water intake (kg/d) by week and treatment (n=72: 18 IN, 18 PN, 18 IB, 18 PB). Data from wk 8 and 9 from calves that were tested in the visual discrimination test (n = 6 per treatment) were excluded. Calves that were reared in pairs with a water nipple enrichment drank more water over time than calves in the other treatments (P=0.001).

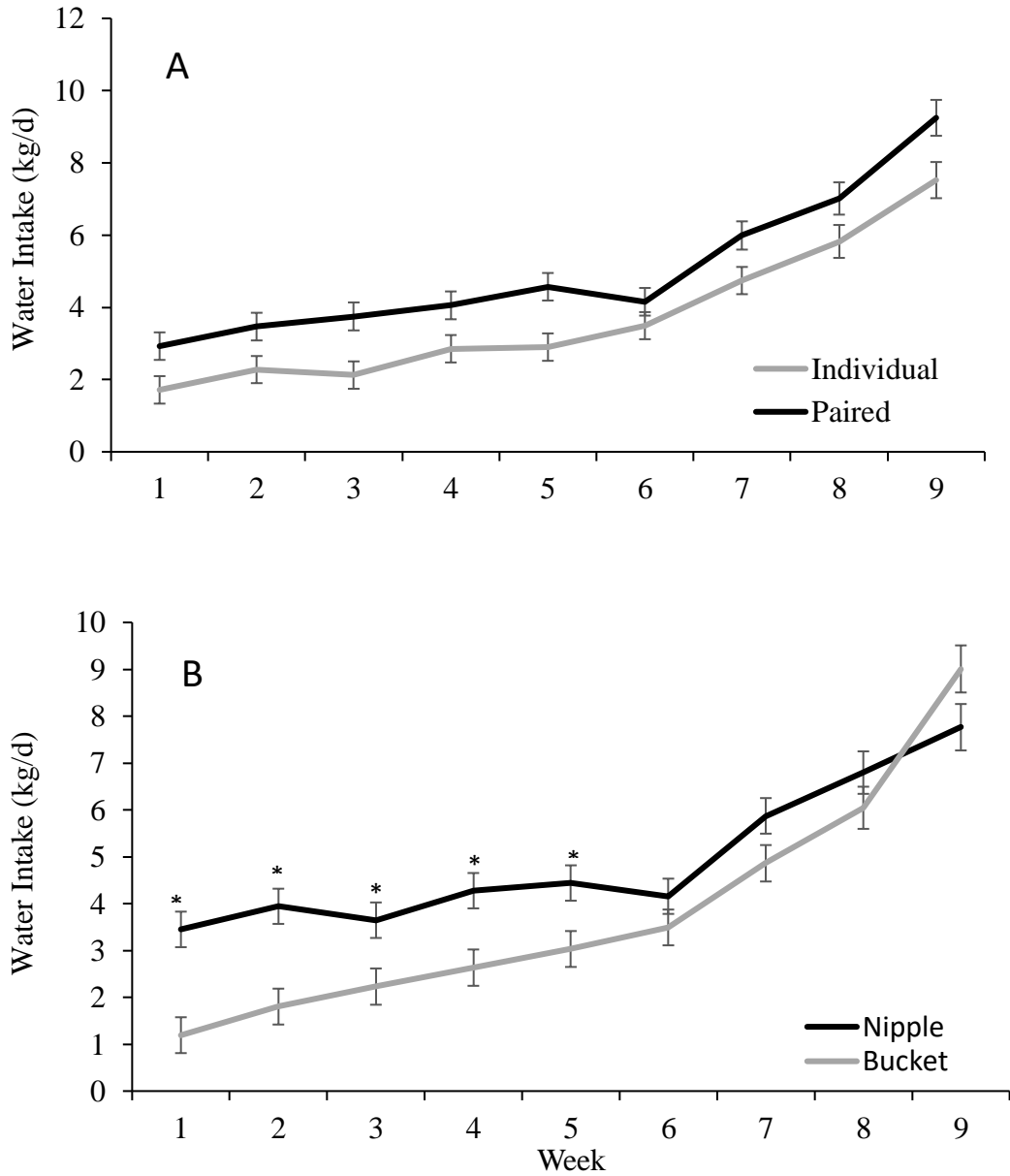


Figure 4. Mean ( $\pm$ SEM) daily water intake (kg/d) by week and enrichment level. Calves enriched socially (n=36) drank more water than calves reared individually (**A**; n=36, P=0.004). There was an interaction between the nutritional enrichment level (bucket: n=36, nipple: n=36) and week (**B**; P<0.0001). Asterisks denote weeks with differing means P < 0.05.

### 3.1.2. Grain Intake

Holstein calves consumed more grain than Jersey calves ( $0.79 \pm 0.04$  vs  $0.51 \pm 0.04$  kg/d;  $P < 0.001$ ). As weeks progressed calves increased their grain intake overall ( $P < 0.001$ ). Overall there was no effect of social or nutritional enrichment on grain intake ( $P > 0.35$ ) and no social by nutritional interaction was detected ( $P = 0.56$ ). For the nutritional enrichment, there was no difference in grain intake between nipple and bucket fed calves across the 9wk period (Figure 5a;  $P = 0.98$ ). However, when considering the social enrichment by week interaction, pair reared calves ate more grain than individually housed calves on wk 8 (Figure 5b;  $1.60 \pm 0.07$  vs  $1.31 \pm 0.07$  kg/d, respectively;  $P = 0.04$ ).

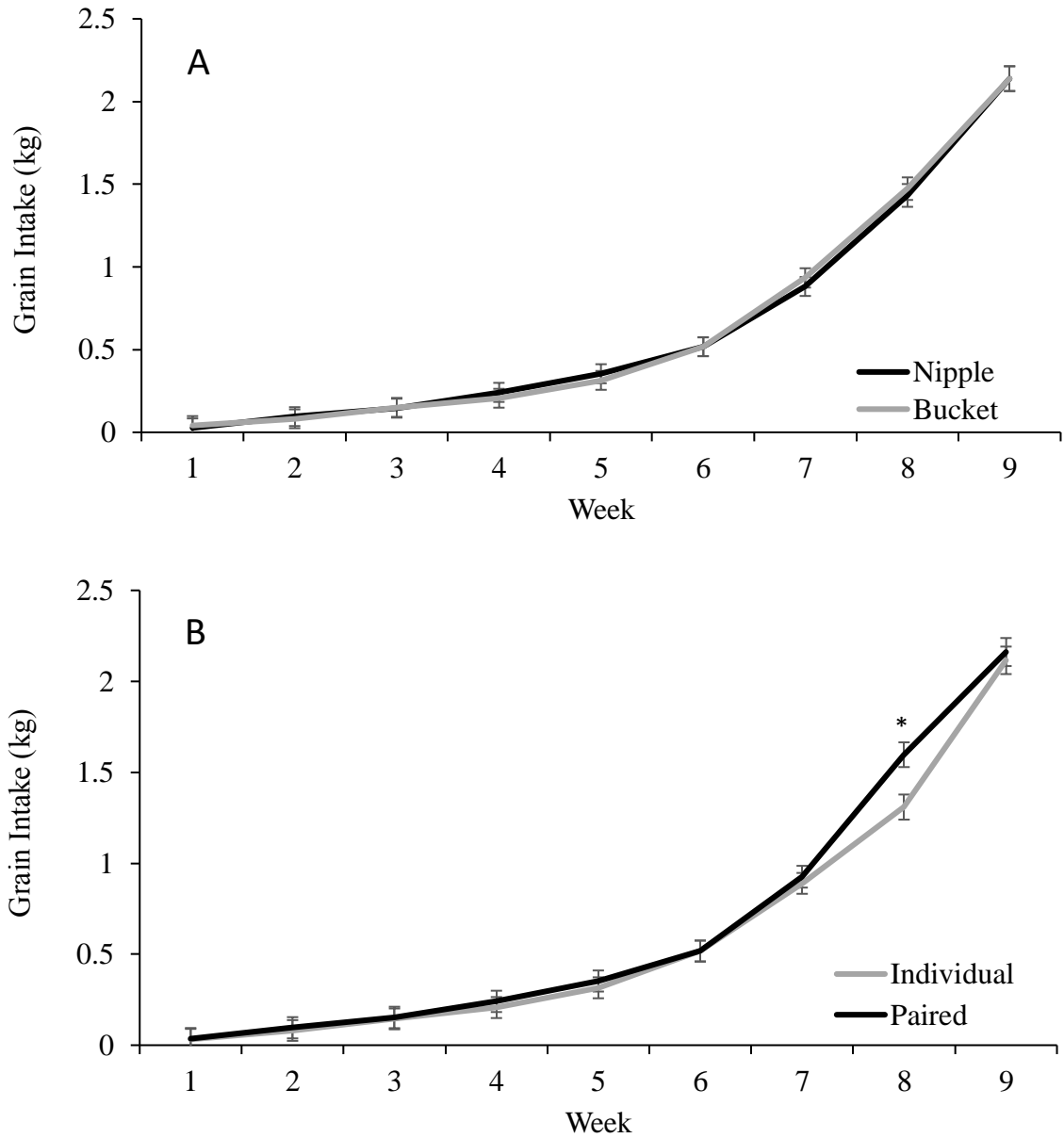


Figure 5. Mean ( $\pm$ SEM) grain intake (kg/d) by week and enrichment level. No difference in grain intake was found between nutritional enrichment levels (**A**; bucket: n=36, nipple: n=36,  $P=0.96$ ). Pair reared calves (n=36) only ate more grain than individual calves only during wk 8 (**B**; n=36,  $P=0.04$ ).



### 3.2 Body Weight

There were no differences in overall body weight among calves in either the social or nutritional enrichment treatments (Figure 6;  $P > 0.20$ ), as well as no treatment by week interaction ( $P > 0.30$ ) or social by nutritional enrichment interaction ( $P = 0.97$ ). A difference breed was found when considering body weight by breed (Holstein:  $59.93 \pm 1.25$  kg vs Jersey:  $45.76 \pm 1.30$  kg;  $P < 0.001$ ). Body weight increased with each week in all treatments ( $P < 0.001$ ).

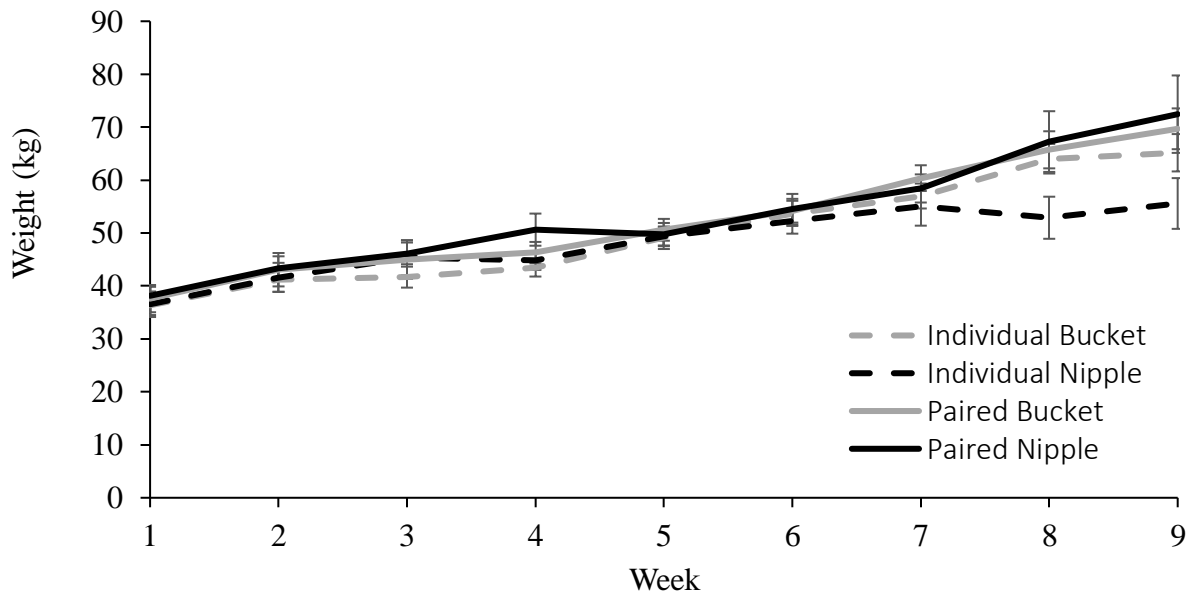


Figure 6. Mean ( $\pm$ SEM) body weight by treatment ( $n=72$ : 18 IN, 18 IB, 18 PB, 18 PN).

Treatment had no effect on body weight ( $P > 0.20$ ). As time progressed body weight increased ( $P < 0.0001$ ).

### 3.3. Behavior Before and After Milk Delivery

#### 3.3.1 Standing

Standing behavior was not a mutually exclusive behavior from the others measured, and therefore includes all behaviors recorded except lying. Before milk delivery, Jerseys stood more than Holsteins ( $17.35 \pm 0.95$  vs  $12.69 \pm 1.02$  min;  $P = 0.004$ ). Nutritional enrichment level was also associated with standing behavior before milk delivery; nipple enriched calves stood less than bucket fed calves ( $13.43 \pm 0.94$  vs  $16.61 \pm 0.99$  min;  $P = 0.03$ ). Enrichment type was not associated with standing time after milk delivery, nor were any other fixed effect or interactions ( $P > 0.06$ ).

#### 3.3.2. Lying Behavior

Pre-milk delivery lying time was higher for Holsteins than Jerseys calves ( $15.70 \pm 0.88$  vs  $12.94 \pm 0.83$  min respectively;  $P = 0.04$ ). Only nutritional level had an effect on lying time post-milk delivery; Calves offered a nipple to drink water from laid for more time than calves offered water buckets ( $9.19 \pm 0.97$  vs  $6.17 \pm 1.02$  min, respectively;  $P = 0.04$ ).

#### 3.3.3. Grooming

Grooming behavior across periods peaked during period 2 for the 30 minutes pre-milk delivery across enrichment levels (Period 1:  $0.91 \pm 0.10$  min, period 2:  $1.13 \pm 0.10$  min, period 3:  $0.70 \pm 0.10$  min,  $P = 0.01$ ). After milk delivery, grooming behavior decreased across periods (Period 1:  $1.31 \pm 0.14$  to Period 3:  $0.78 \pm 0.14$  mins;  $P = 0.03$ ). By breed, grooming was performed more by Jerseys than Holsteins ( $1.31 \pm 0.12$  vs  $0.81 \pm 0.13$  min

respectively;  $P = 0.01$ ). Only the nutritional enrichment had an effect on grooming time post-milk delivery; calves reared with water buckets groomed more than calves reared with water nipples (Figure 7;  $1.24 \pm 0.13$  vs  $0.89 \pm 0.12$  min, respectively;  $P = 0.05$ ).

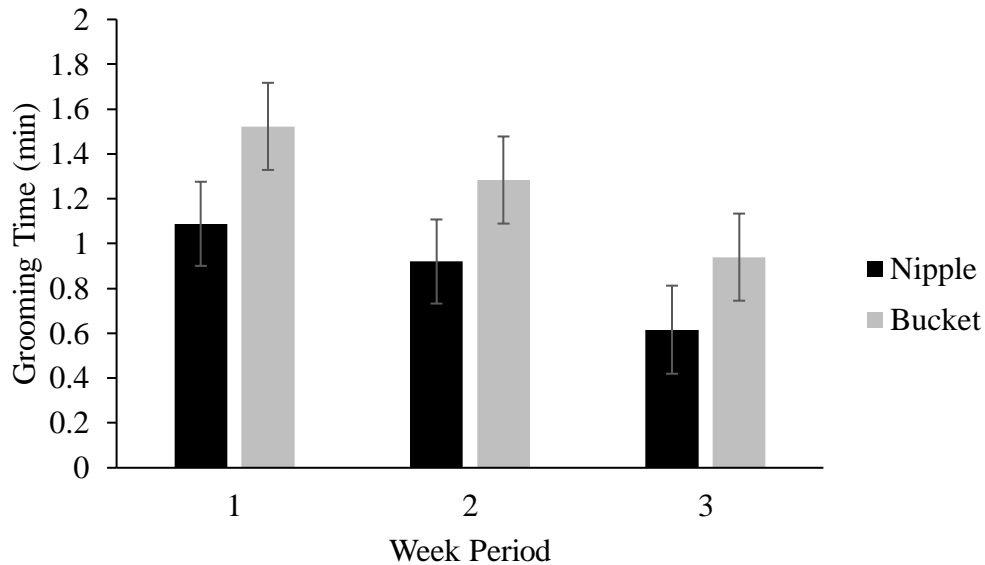


Figure 7. Mean ( $\pm$ SEM) grooming time by nutritional enrichment level in the 30 min post-milk delivery. Calves reared with a water nipple enrichment ( $n=14$ ) groomed less when compared to calves reared with water buckets ( $n=12$ ;  $P = 0.05$ ). As time progressed calves spent less time grooming ( $P = 0.03$ ).

#### 3.3.4. Eating Grain

No variable or interaction effects were found during the 30 minutes pre-milk delivery ( $P > 0.12$ ). Post milk delivery, eating time increased with each week period (period 1:  $0.80 \pm 0.21$  min, period 2:  $1.02 \pm 0.19$  min, period 3:  $1.56 \pm 0.19$  mins;  $P = 0.03$ ). Holsteins visited the grain bucket more than Jerseys ( $1.49 \pm 0.16$  vs  $0.760 \pm 0.16$  min;  $P = 0.004$ ). No other variable or interaction effects were found ( $P > 0.06$ ).

### 3.3.5. Drinking Water

No variable or interaction effects were found in the 30 minutes pre-milk delivery ( $P > 0.20$ ). After milk delivery, water drinking behavior differed by nutritional enrichment level; calves offered a water nipple drank longer than calves offered a water bucket (Figure 8;  $2.62 \pm 0.25$  vs  $0.31 \pm 0.28$  min, respectively;  $P < 0.001$ ). Pair reared calves also drank longer overall than individually reared calves ( $1.89 \pm 0.27$  vs  $1.04 \pm 0.28$  min, respectively;  $P = 0.04$ ). Calves enriched with a water nipple performed the drinking behavior more compared to calves provided water from a bucket, when also pair reared (Figure 8;  $P = 0.04$ ).

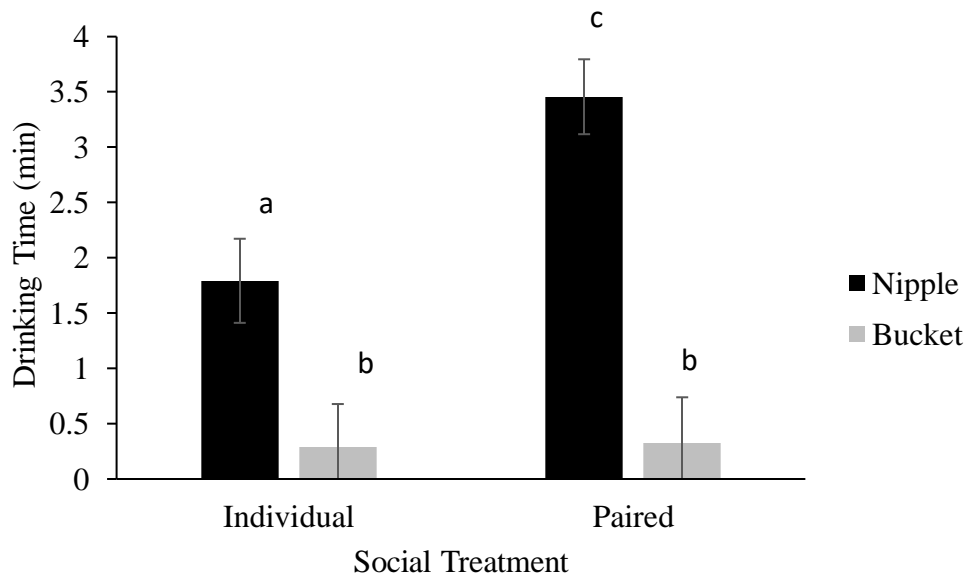


Figure 8. Mean ( $\pm$ SEM) drinking time for calves in the nutritional enrichment treatment (nipple:  $n=14$ , bucket:  $n=12$ ) and social enrichment treatment (paired:  $n=14$ , individual:  $n=12$ ) during the 30 min post-milk delivery. Means with different letters differ ( $P < 0.05$ ).

### 3.3.6. Drinking Milk

Milk drinking behavior was performed more by Jerseys than Holsteins ( $6.97 \pm 0.41$  vs  $5.53 \pm 0.41$  min, respectively;  $P = 0.02$ ), and by water bucket fed calves compared to water nipple fed calves ( $7.13 \pm 0.40$  vs  $5.37 \pm 0.39$  min;  $P = 0.005$ ). No other variable or interactions were found ( $P > 0.20$ ).

### 3.3.7. Non-nutritive Suckling

In the 30 min pre-milk delivery period, Jerseys performed non-nutritive suckling more than Holsteins ( $5.98 \pm 0.52$  vs  $2.09 \pm 0.55$  min, respectively;  $P < 0.001$ ). No difference was found between individual and pair rearing, but when calves were reared with a water bucket and partner non-nutritive suckling behavior decreased (Figure 9;  $P = 0.05$ ).

Non-nutritive suckling during the 30 min after milk feeding, increased as the calf got older ( $P = 0.03$ ). Jerseys performed more non-nutritive suckling than Holsteins ( $4.74 \pm 0.45$  vs  $2.52 \pm 0.48$  min, respectively;  $P = 0.003$ ).

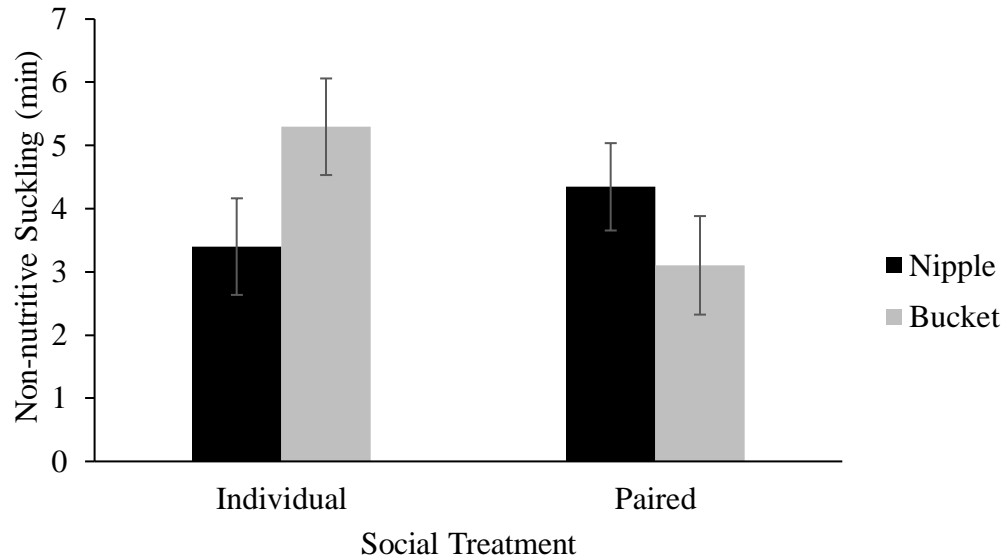


Figure 9. Mean ( $\pm$ SEM) non-nutritive suckling time by nutritional enrichment level (nipple: n=14, bucket: n=12) and social enrichment level (paired: n=14, individual: n=12) during the 30 min post-milk delivery. No effect differences were detected between the for treatment levels ( $P > 0.06$ ).

### 3.3.8. Cross Suckling

No variable or interactions were found in the 30 minutes pre-milk delivery ( $P > 0.08$ ).

Cross suckling behavior post milk delivery increased over time ( $P = 0.03$ ) and for calves fed water from a bucket, but not for calves fed water from a nipple (Figure 10;  $P = 0.05$ ).

A week by nutritional enrichment interaction was found to show cross suckling to increase for water bucket calves over time but not for calves enriched with a water nipple (Figure 10;  $P = 0.02$ ).

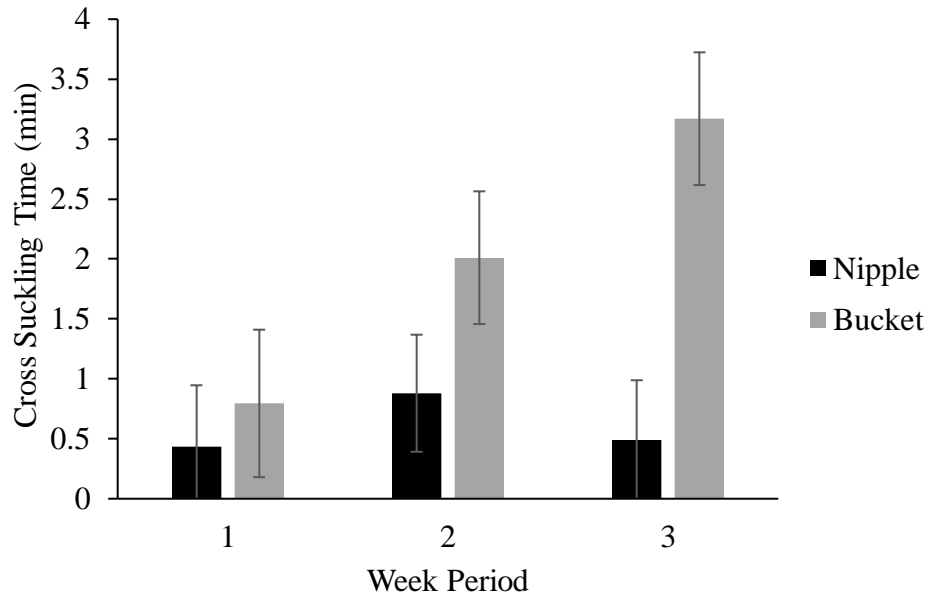


Figure 10. Mean ( $\pm$ SEM) cross suckling by nutritional enrichment level (nipple: n=14, bucket: n=12) during the 30 min post-milk delivery. The largest difference in time spent cross suckling occurred during week period 3 ( $P=0.02$ ).

### 3.4 Visual Discrimination Test

Calves increased in the percent correct choices throughout sessions, however, calves on average did not perform better than chance (50%) until the 8<sup>th</sup> session (Figure 11;  $P < 0.001$ ). When considering the social enrichment, overall, individually reared calves performed better than pair housed calves throughout the visual discrimination task (Figure 11a;  $0.63 \pm 0.02$  % correct/total choices vs  $0.57 \pm 0.02$  % correct/total choices respectively;  $P = 0.05$ ). Nutritionally enriched calves offered water nipples chose, overall, more correct choices than non-nutritionally enriched bucket calves (Figure 11b;  $0.64 \pm 0.02$  % vs  $0.56 \pm 0.02$  %,  $P = 0.02$ ). IB and PB calves reached the learning criterion with a median of 17 sessions (range 14-17, 17 respectively), IN calves at 10.5 sessions (8-

14) and PN calves at 15.5 sessions (10-14). IN calves compared to IB and PB treatments had fewer sessions until learning was achieved ( $P < 0.05$ ); while PN calves were quicker to reach learning than PB calves ( $P < 0.05$ ).



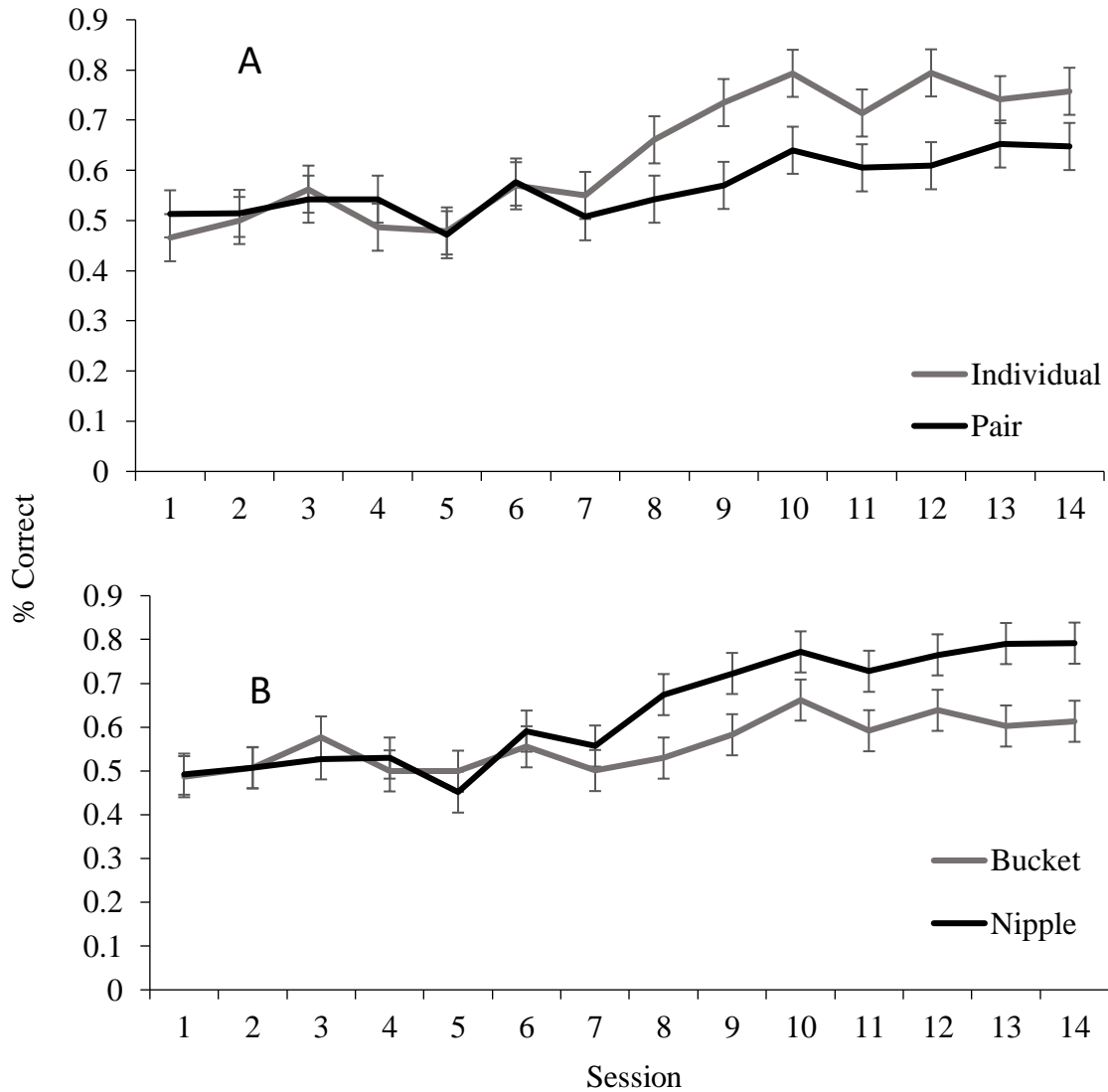


Figure 11. Mean ( $\pm$ SEM) correct responses for social enrichment treatment (**A**; individual: n=6, paired: n=6) and nutritional enrichment treatment (**B**; nipple: n=6, bucket: n=6) during the 14d visual discrimination task. Calves reared with a water nipple enrichment had higher overall percent correct choices than calves reared with a water bucket ( $P=0.02$ ). Calves that were reared in pairs had fewer overall percent correct choices than calves reared individually ( $P=0.05$ ).

## 3.5 Discussion

### 3.5.1 Grain intake, water intake, and body weight

It was predicted that calves reared with both the social and nutritional enrichment would have greater grain and water intake resulting in higher body weights. Previous research has shown the positive effects of social enrichment around weaning; however, no research has followed calves feed intake and body weight when offered two forms of enrichment throughout development until weaning.

Holstein calves consumed about 0.28 kg/d more grain than Jersey calves. This difference is likely due to the difference in breed size and body weight (Dhakal et al., 2013). During wk 8, paired reared calves ate more grain than individual reared calves. The final step of weaning (no milk) occurred during wk 8 and paired calves may have responded better to the weaning process (greater grain intake) due to being in a positive affect state from the presence of their social partner. Paired calves likely experience a positive affect state because of the presence of a familiar calf, which acts as a stress buffer (Heinrichs et. Al., 2004) and source for positive social interaction, which has been found to suppress the hypothalamic pituitary adrenal axis through mechanisms that involve oxytocin release via contact (DeVries et. Al., 2007). Social learning has been shown to increase grain intake post weaning (Miller-Cushon and DeVries, 2016; De Paula Vieira et al., 2010) as paired calves have access to twice the amount of relevant information than do individually reared ones through observation of their partners' successes and failures

(Templeton and Giraldeau, 1995; 1996). As calves transitioned fully from a milk based diet to grain based diet they depended more on social learning by mimicking feeding behaviors they saw their partners performing (Miller-Cushon and DeVries, 2016).

Water intake was on average 1.3 kg/d greater for calves raised in pairs versus individually. The effect of social enrichment may be due to the gregarious nature of cows. Since calves are herd animals, they form preferential social bonds during various behavior activities (Gygax et al., 2010; Reinhardt and Reinhardt, 1981), which could be the reason for the increase in water drinking behavior in pair reared calves also reared with a water nipple compared to the individual treatments. Water intake was on average 1.1 kg/d greater for calves raised with a water nipple over bucket. An increase in water intake for calves reared with a water nipple is likely a redirection of the suckling behavior onto the water nipple. Amongst calves housed individually there was little difference in water intake between nutritional enrichment level; however, calves housed in pairs with the water nipple enrichment drank more than the pairs reared with water buckets. The combination of both social facilitation, from the partner drinking water, and motivation to suckle were highly effective at increasing water intake. In this case, it is likely the auditory noise of the partner calf suckling on the water nipple that encouraged the focal calf to drink as well. Across time, calves fed water from a nipple consumed more water than calves fed water from a bucket until week 6 after which there was no difference. This interaction suggests that calves reared with a water nipple were affected by weaning in regard to water intake; however, since there were fewer

milk delivery periods, there were fewer opportunities to displace suckling behavior on the water nipple thus resulting in lower water intake for the period surrounding weaning.

The prediction that an increase in water intake would yield an increase in grain intake was not supported in this study, perhaps because there are other factors that contribute to this relationship, like enhanced milk allowance (Jensen et al., 2015).

Jersey calves ate less than Holstein calves and had lower body weights across the weeks, to be expected as they are the smaller of the two breeds (Dhakal et al., 2013). Body weight was not affected by treatment level.

### 3.5.2 Behavior Before and After Milk Feeding

Behavior data were only collected for the 30 min before and after the P.M. milk delivery. This period was selected because oral behaviors (grooming, drinking, cross and non-nutritive suckling) are performed shortly before or after milk delivery and would give insight on the utilization of the two enrichment types (Lidfors, 1993). Milk delivery was broken up into two periods, the 30 mins before milk delivery (pre-milk delivery) and the 30 mins after milk delivery (post-milk delivery). Pre-milk delivery showed anticipatory behaviors (behaviors performed in anticipation to milk delivery and consumption) while post-milk delivery showed displacement (suckling behavior performed with/on alternative source) and redirected behaviors (alternative behavior

performed to cope with the inability to perform suckling). This section will focus first on all significant behaviors performed during the pre-milk period and then on all significant behaviors performed during the post-milk period.

#### 3.5.2.1 Pre-milk delivery

Calves present anticipatory behaviors in a variety of ways, from increased locomotion to oral displays. These behaviors are likely expressed because the calf is motivated by the arrival of a stimulus or frustrated from the lack of the anticipated stimulus.

Pre-milk delivery, nutritionally enriched water nipple calves stood less than calves reared with water buckets ( $8.43 \pm 0.77$  vs  $10.76 \pm 0.81$ min) as they had lower feed anticipatory behavior (Mistlberger, 2011; de Paula Vieira et al., 2008). This anticipatory feed behavior in calves reared with a water bucket is likely due to a higher motivation for milk delivery (in regards suckling ability), since they lack a nutritional form (water nipple) of suckling in their rearing environment.

Grooming behavior was highest before milk delivery during wks 3, 4, and 5 which encompassed the first step down in weaning (two 5L bottles of milk to one 5L bottle/d; only P.M. milk delivery). Increased anticipation of P.M. milk delivery (because calves did not receive A.M. milk) resulted in a disproportionate increase in self-grooming as coping mechanism to self sooth (Krohn, 1994) among all treatments.

Individually reared calves with a water bucket had longer non-nutritive suckling times compared to individual calves provided water from a nipple; this relationship was reversed when calves were paired in each treatment, whereby non-nutritive suckling was higher in paired calves provided a water nipple versus a water bucket. These results conflict with the prediction that calves reared with a water nipple would displace suckling behavior on the water nipple and thus perform less non-nutritive suckling. Perhaps paired nutritionally enriched calves had attempted to redirect anticipatory suckling behavior on the water nipple and were frustrated that they received water instead of milk causing them to seek alternative sources for milk, which yielded greater non-nutritive suckling.

Pre-milk delivery, there appeared to be an increase in anticipatory behavior for calves reared with water buckets in the form of standing; these calves were more motivated to suckle due to the lack of a suckling stimulus in their rearing environment. Paired calves reared with water nipples expressed their anticipatory behavior in the form of frustration by performing non-nutritive suckling, likely because when they redirected anticipatory suckling behavior when they were not rewarded with milk after first displacing suckling on the water nipple. Overall, the first step-down in weaning presented an increase in grooming behavior among all treatments, which could be indicative of the stressful effect of weaning.

### 3.5.2.2 Post-milk delivery

The post-milk delivery period highlights issues of suckling behavior that the calf was unable to complete during milk delivery. Nutritive suckling of milk lasts for approximately 23 min in dam-reared calves, while in conventional settings it lasts 10.6 min for ad libitum milk-fed calves and for 7.25 min for restricted milk-fed calves. The 3 min discrepancy between conventional milk feeding systems doesn't allow restricted milk-fed calves enough time for negative feedback (Cholecystokinin secretion) from the meal to take effect, and results in calves exhibiting frustration from hunger via redirected or displaced suckling behavior (De Paula Vieira et al., 2008; Veissier et al., 2013). Abnormal suckling behaviors (cross- and non-nutritive suckling) are largely exhibited post-milk delivery as there is strong motivation to feel satiated on a restrictive milk allowance, which typically occurs 10 min post-milk delivery when CCK secretion has gone into effect (de Passillé et. al., 1992).

Negative oral behaviors like cross and non-nutritive suckling are performed more when calves are attempting to compensate for the inability to perform the suckling behavior or obtain access to milk (Loberg and Lidfors, 2001). Time spent performing the cross-suckling behavior increased during the study as weeks progressed for calves reared on water buckets and stayed constant for calves reared on water nipples. The suppression of oral activities like suckling among calves reared with a water bucket appears to increase cross-sucking, an abnormal oral behavior (Jung and Lidfors, 2001; Fraser and Broom, 1990). Provision of a nutritional water nipple enrichment decreases non-

nutritive suckling after milk-delivery as calves satisfy the motivation to suckle by displacing the behavior on the water nipple (de Passillé, 2001). Similarly, non-nutritive suckling increased over time across treatments, possibly due to the confounding effects of a restricted milk diet and weaning.

Calves reared with a water bucket took longer to consume milk than calves enriched with a water nipple ( $7.13 \pm 0.40$  vs  $5.37 \pm 0.39$  min, respectively) in the 30 minutes post-milk delivery. This is a particularly interesting finding, as calves from all treatments had access to milk bottles and the same quantity of milk at each feeding period. Ideally milk bottles were pulled by staff as soon as they emptied to reduce sucking in air and causing bloat, however they were occasionally left longer which allowed calves to suckle on the milk bottle nipple until its removal. While the fullness or emptiness of the milk bottle could not be determined from the camera footage, it's hypothesized that part of the increase in milk drinking time for calves reared with a water bucket is due to this. In human infants the act of tongue- and jaw-lowering movement plays a primary role in increasing sucking strength (Tamura, 1998); perhaps the lack of suckling muscle stimulation among calves reared with a water bucket resulted in an increased time to suckle milk.

Water drinking time post-milk delivery supports the idea of displacing an unfinished suckling behavior on the water nipple enrichment, as time spent drinking water was greater for calves offered water from a nipple (on average 2.31 min longer). Calves



reared in pairs exhibited 0.85 min longer water drinking times compared to individually reared calves, likely due to social facilitation. Time spent eating increased as the week's progressed, post-milk delivery, showing that calves in this study investigated and preferred to eat grain after milk was delivered, rather than before.

Post-milk delivery, grooming decreased throughout the week. Calves reared with a water bucket performed the grooming behavior more often than calves reared with a water nipple, perhaps because they are less satisfied orally or stressed (Krohn, 1994) from the inability to perform the suckling behavior to satiation. Water bucket reared calves also spent less time lying when compared to water nipple calves post-milk delivery ( $6.17 \pm 1.02$  min vs  $9.19 \pm 0.97$ , respectively) indicated they were standing more after milk-delivery.

### 3.5.3 Visual Discrimination Task

Contrary to results found by Gaillard et al. (2014), individually reared calves performed better throughout the visual discrimination task than pair reared calves. One notable difference between the two studies was housing: pair reared calves were grouped in one large hutch together and therefore had full contact with each other (Gaillard et al., 2014). In our study, paired calves experienced partial paired rearing with limited physical contact through a fence as they were reared in individual hutches. The setup of our study did not replicate environment designed in the previous publications; however, this does not invalidate our results. Many producers (~60%) are still reluctant to house

calves together and instead opt for systems that keep calves in close proximity to each other (i.e. tactile and visual contact) yet still in their own pen so that they can be fed and managed individually (USDA, 2014). In our study there appeared to be an advantage to individually rearing calves, as paired calves had fewer overall correct choices during the visual discrimination test. One explanation may be the increased motivation for individually reared calves to find milk; these calves were socially limited and had few stimulating experiences throughout their day. Arguably the most stimulating experience individually reared calves had was milk delivery, and in the same respect may have been more motivated to find milk to perform the suckling behavior. Water nipple reared calves, overall, chose more correct choices than bucket fed calves, aligning with the original hypothesis that enriched calves would perform cognitive tasks better than non-enriched calves (Gaillard et. al., 2010; Meagher et al., 2015). This difference could be due to the calf's familiarity with nipples, however all calves experienced the milk nipple similarly, so it is less likely that is the case. It is more likely that the nutritional water nipple enrichment allowed calves to satisfy their motivation to suckle offering environmental complexity (de Passillé, 2001). Deprivation of early environmental stimulation has been associated with decreased development in neural cognitive functioning through disrupting inhibitory control in attentional selection (Wurbel, 2001). Early life exposure to enrichment may support early cognitive development, and the ability to learn in dairy calves. As calves grow, they face environmental and management changes with stressors like regrouping, and novel environments such as the milking parlor. Their ability to habituate, learn and adapt to these situations is

critical to improving welfare. Further research needs to be done on the long-term effects of early life enrichment exposure to measure the true potential and benefits on dairy cows' welfare.

### 3.6 Conclusion

While incorporation of both a social partner and water nipple enrichment were only found to have additive effects on water intake and water drinking time, the effects of each enrichment independently influenced cognitive success in a visual discrimination test. Water intake was greater for nutritionally enriched nipple fed calves and effected accompanying behaviors (increase in water drinking time, decrease in milk drinking time and a decrease in standing time). This indicates that the presence of a water nipple allowed the calf to displace the suckling behavior around milk delivery resulting in reduced frustration. Socially enriched calves were also found to have a greater water intake, increased time spent drinking and greater grain intake during the final stage of weaning, indicating positive advantages for pair rearing also. Cross-suckling, an abnormal oral behavior, was greatly reduced across 9 wks when calves were nutritionally enriched with a water nipple while calves lacking the nutritional enrichment increased time spent performing the behavior across time. The benefit to being pair reared in the non-nutritionally enriched calves was a decrease in non-nutritive suckling post-milk delivery. Both the nutritional and social enrichment showed a combined effect that positively increased water intake and drinking time overall. Calves were able to learn a simple cue discrimination task, where nutritionally enriched

nipple fed calves and individually reared calves had a higher success at overall correct choices. Incorporating a water nipple enrichment into existing calf rearing systems may improve the calf's ability to learn new a task and express a highly motivate behavior (suckling) thereby improving overall calf welfare.

## Chapter 4

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