

# **Behavioral Biomarkers for Calf Health**

by

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## **Abstract**

Applied ethology is a diverse scientific field studying animals in confinement under human management. Data collection techniques including automated measures (e.g. activity monitors and environmental enrichment devices) and video recording systems aid in collecting animal behavior data while reducing more invasive collection measures. Understanding early life behavior's in both dairy and beef calves is important for the health, performance, and welfare of these animals. Oral behaviors early in life can affect a calf's performance and health through adulthood. Applied ethologists utilize automated technologies to better quantify the behaviors of dairy and beef calves early in life which could result in changes in management of calves.

In the first study, the objectives were to validate an environmental enrichment device for individually housed Holstein dairy calves and describe methods for behavioral data collection. Holstein bull calves were fitted with 3-axis accelerometers (activity monitor) and provided an environmental enrichment device. A total of 59 h of video footage was analyzed for calf behaviors. Observed EED use was shown to be highly correlated with automated EED. Observed standing and lying durations were correlated with automated standing and lying durations. Use of environmental enrichment can aid in collection of data and allow animals to express natural behaviors such as suckling. Furthermore, data collected through automated techniques can give valuable knowledge without increasing distress to animals and providing more time efficient methods of data collection.

In the second study, the objective was to determine if calf behavior data collection techniques (both direct observation and automated) would correlate with measures of passive transfer of immunity (hematocrit; total plasma protein; Immunoglobulins, IgG1 and IgM). Variables collected included: each calf's first-meal suckling behaviors; automated activity

behaviors, and measures of passive transfer of maternal antibodies (IgG1, Igm, hematocrit, and total plasma protein). There was a tendency for IgG1 concentrations to have an inverse relationship with calf body weight. Total plasma protein was correlated with IgG1 and IgM. Likewise, hematocrit was correlated with IgG1 and IgM. Total Plasma Protein had an inverse relationship with birth-to-stand intervals. In addition, total plasma protein tended to decrease as the birth-to-suck interval increased. Total plasma protein increased with the number of lying-bouts per day for the first week of life. Furthermore, there was a tendency for the birth-to-last teat suckled to decrease as the temperature on the day of birth increased. Calf lying duration during the first week of life increased as the temperature on the day of birth increased. These data demonstrate calf behaviors early in life are associated with measures of passive transfer of immunity.

Overall, these data support the use of applied ethology techniques including live/video observation and automated data collection to help address early life behaviors in both dairy and beef cattle. Automated technologies such as an environmental enrichment device could help collect important sucking behavior data while providing dairy calves an avenue to satiate their motivation to suck. Furthermore, observed behavioral measures such as the birth-to-stand measure in beef calves, can give an indication to their passive transfer of immunity status. Future research in regards to animal behavior could benefit from the use of video recording systems and automated technologies resulting in better management of animals under human care.

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# Chapter 1 - Literature Review

## Introduction

Behavior for animal science includes a combination of behaviorism, ethology, and applied ethology, although all three fields emphasize using animal behavior as an important measure for understanding the proximate (i.e. physiology), ontogeny, phylogeny, and function of animals. Ivan Pavlov, B. F. Skinner, and John B. Watson were pioneers of behaviorism and believed all behavior is caused by external stimuli, emphasizing the role of ontogeny and environment (Sapolsky, 2017). This area of study focuses on learning and more specifically behavior from operant and classical conditioning (learning theory). The challenge with behaviorism methodologies is that animals (typically rodents and dogs) are placed in test arenas or artificial environments. In contrast, ethologists study animals' behavior under natural conditions. By focusing on animals in their natural environment, ethologists study an animal's typical behavior (instinctual) which may be diminished in a restricted environment (i.e. laboratory, test arenas). Karl von Frisch, Konrad Lorenz, and Nikolaas Tinbergen are considered the founders of Ethology (Veissier and Forkman, 2008). The field of applied ethology is a combination of behaviorism and ethology; Applied ethology is the study of animal behavior in relation to animals under human management (i.e. homes, farms, production, and zoos). This scientific field focuses on better understanding animal behavior to improve management and quality of life (e.g. animal welfare). The most well-known applied ethologist is Dr. Temple Grandin and her research with cattle handling facilities and welfare (Grandin, 2014). The field of applied ethology is important for understanding behaviors displayed by livestock. The focus of this thesis is to better understand oral behaviors performed by beef and dairy calves and early life

activity. For this thesis, the proximate, ontogenetic, functional, and phylogenetic components of oral behaviors in calves will be considered (Figure 1).

## **Proximate**

### ***Digestion system in Ruminants***

Although cattle are typically differentiated from other livestock species because they are foregut fermenters, ruminant species start life with a monogastric-like digestive system to efficiently absorb milk. Most of a ruminant-neonate's digestive system is made up of the abomasum, which is very similar to a monogastric stomach, the other three compartments (i.e. reticulum, rumen, omasum) are underdeveloped during the suckling phase. They start developing after 2 weeks of life, when the calf starts to consume solid feed that enters the reticulum and rumen. A special anatomical structure known as the esophageal groove forms when a calf begins to suckle. The suckling reflex causes two muscular folds (from the reticulorumen) to close creating a bypass for colostrum and milk into the abomasum of the neonate (Comline and Titchen, 1951). A clot is formed as the milk settles in the abomasum and is broken down by enzymes called pregastric esterase in neonates (Huber et al., 1961). These pregastric esterases are stimulated by the nursing in the young calf (Huber et al., 1961; Moreau et al., 1988; Ramsey and Young, 1961). Other digestive enzymes remain limited in the digestive system to allow macromolecules, including Igs, to not be digested and instead absorbed (Thivend et al., 1980).

### ***Significance of the first meal among ruminants***

An efficient first meal is especially important among ruminant neonates due to the fact they receive no maternal antibodies through the placenta. The placenta of the cow forms a syncytium between the maternal endometrium and fetus resulting in a separation of the maternal and fetal blood supplies, preventing transfer of immunoglobulins to the fetus (Arthur et al.,

1996). All of the antibodies a ruminant neonate receives are post digestion of their first meal of colostrum. The dam begins to mobilize immunoglobulins, primarily IgG1 and IgM, into the mammary gland where it is then passed to the neonate upon suckling. These large molecules are non-selectively absorbed for a short period of time through the stomach epithelium and into the bloodstream by a mechanism known as pinocytosis. The absorption of these large molecules is non-selective for a short period of time decreasing through day 7 postnatally and replaced with digestion of proteins in the gastrointestinal tract lumen and enterocyte borders (Blum, 2004). Tight junctions in the epithelium of the neonate gut begin closing within 4 hours after birth (Stott et al., 1979). The absorption of immunoglobulins is highest within those 4 hours after birth and decreases significantly after 12 hours (Weaver et al., 2000). Calves fed earlier have a significantly higher concentration of IgG in serum than calves fed later (Stott et al., 1979).

In addition, calves receive other important nutrients from colostrum besides immunoglobulins. Colostrum of the dam contains proteins, fats, vitamins, and minerals necessary for survival of the calf. Many of these vitamins and minerals are supplemented to the dam in order to have an adequate amount in the colostrum ingested by the calf (Weiss et al., 1992; Kume and Tanabe, 1993; Swecker et al., 1995). Lipids in colostrum provide neonatal calves with their first energy supply which is important for metabolic function. The lipid component of the colostrum is needed for the calf to stand and generate body heat if born in a cold environment. Furthermore, proteins including Igs are available to the neonate calf for amino acid synthesis and gluconeogenesis. Proper oral behaviors are important for calf health immediately after birth and extend to lifelong performance in production.

### **Ontogeny**

### *Oral behaviors in-utero*

Initial development of oral behaviors can be seen prior to a neonate being born. Human and ovine fetuses express amniotic swallowing in the latter parts of gestation (Pritchard 1965; Bradley and Mistretta, 1973 and 1975; Abramovich et al., 1979; Tomoda et al., 1985). Fetal swallowing expression is related to hypoxia, hypotension, and fluid osmolality which could be an indication of the quality of the dam's environment. This non-nutritive oral behavior is related to the amount of amniotic fluid around the fetus and is thought to allow regulation of the amount of total amniotic fluid. Nonetheless, if amniotic fluid volume is above normal, fetal swallowing was increased compared to ovine-fetuses with normal or low amniotic fluid volume. The reduction in fetal swallowing (daily swallowed volume and volume per swallow) has been shown to occur when amniotic fluid volume was 38% lower than normal (Kullama et al., 1994). Kullama and others drained amniotic fluid and fetal urine, with use of catheters, in ovine fetuses resulting in a reduction in fetal swallowing. Increased fetal swallowing was observed when higher than normal volumes of amniotic fluid were present in ovine fetuses but no significant change in volume swallowed was observed (Brace et al., 2014). The act of fetal swallowing may aid the dam in amniotic fluid homeostasis and potentially prepare the calf for suckling after birth. Development of amniotic swallowing was thought to be from changes in the fetuses taste perception due to the amniotic fluid composition (Bradley and Mistretta, 1973). However, more recent research did not replicate this observation with intra-amniotic infusions (Brace and Cheung, 2004; Robertson et al., 2009). Expression of amniotic swallowing pre-partum may indicate importance of non-nutritive oral behaviors post-partum and in the early weeks of life.

Oral behaviors including non-nutritive behaviors early in life are important for calf development. During weaning periods calves can display many oral behaviors especially if

weaned early. The average weaning age of dairy calves in the United States is 8.2 weeks (USDA, 2008) which is significantly lower than the age of weaning in the wild (6 months) (Phillips, 2001). Development of abnormally expressed oral behaviors has been linked to early weaning (Latham and Mason, 2008). Calves transition from a milk diet originating from their dam's udder or bottle in the case of dairy production, to a grain and forage diet. A calf must learn to properly eat grain and forage similar to having to learn to suckle properly in order to ingest nutrients. Calves that do not develop proper eating behaviors prior to weaning may continue to perform non-nutritive oral behaviors in an unwanted manner and are at risk for an underdeveloped rumen. Consumption of substrates such as starter and forage allow for the development of the rumen by providing it with fermentation products. Before weaning takes place calves begin to express non-nutritive oral behaviors such as nibbling on substrates in their home environments. This type of non-nutritive behavior is important for proper grazing/foraging skills needed after weaning and the rest of their lives. Calves that were allowed to suckle their dam tend to spend less time on non-nutritive oral activities than calves who were not allowed to suckle their dams (Veissier et al., 2013). Providing an artificial teat after a milk meal or feeding calves through an artificial teat may prevent cross-sucking behaviors (Passillé, 2001).

Common weaning strategies for dairy calves include the restriction of milk until adequate grain or concentrate consumption occurs to wean the calf fully off milk. In order to limit stress a calf experiences it is recommended to do a gradual weaning or step-down method of milk removal rather than an abrupt method (Lidfors, 1993; Nielson et al., 2008). Studies have shown increasing milk and energy intake can reduce non-nutritive sucking and cross-sucking in calves (Jung and Lidfors, 2001; Roth et al., 2009). However, allowing calves ad libitum milk before weaning may result in more difficult weaning due to decreased intake of solid food (Terre et al.,

2007). Passillé and others (2010) found that early weaning did increase postweaning energy intake, but the total intake was below preweaning values. Lower energy intake postweaning could account for the high incidence of cross-sucking after weaning showed by Passillé and others. In addition, they weaned calves at 6 weeks of age compared to 8.2 weeks in North America. Early weaning has been shown to increase non-nutritive behaviors such as cross-suckling in calves. Implications from longer weaning and presence of the dam could help not only with consumer perception of calf welfare but aids the producer in decreasing unwanted behaviors.

### **Functional**

#### ***Abnormal, Stereotypical, and Stereotypies***

Oral behaviors from a functional standpoint can be related to welfare and categorized as normal, abnormal, stereotypical, and stereotypies (Figure 2). In dairy production, abnormal oral behaviors may arise because calves are typically separated from the dams just after the calves acquire their first meal. Non-nutritive oral behaviors are normal for all animals to express, but they are considered abnormal or stereotypies when the behaviors are excessive and cause damage to the individual or pen mates. Behaviors can be abnormal for numerous reasons including: when they differ from normal because they are directed toward inappropriate objects, when they differ from the animal's range of behavior in its nature or frequency, or when they have no function and are harmful to the individual (Mason, 1991; Garner, 2005). Dairy calves are individually housed in hutches or pens with little access to other calves. Abnormal behaviors are expressed by animals in an inappropriate captive environment (Mason, 1991) and generally increase with the lack of socialization (Veissier et al., 1998). This practice is in part largely to the fact calves will suck on each other's navels, genitals, ears, and tail (i.e. cross-sucking) increasing

the risk of disease spreading and injury. The manipulation of different substrates by calves with their mouths is normal exploratory behavior but can be abnormal if it affects their feeding. Incidences of cross-sucking were greater among calves not allowed to suckle their dams (1.8 events/day) than for calves that suckled their dams for 15 minutes post-milking (0.33 events/day). In this study cross-sucking was directed at inguinal region (78%), ear (8%), mouth (6%), throat (3%), naval (2%) and other areas (4%) (Margerison et al., 2003). Non-nutritive oral behaviors such as self-grooming, tongue playing, and cross-sucking occurs in calves fed by bucket and/or housed individually (Pempek et al., 2013). In addition, cross-sucking was shown to be associated with reduced body weight at weaning (Mahmoud et al., 2016). Calves that perform cross-sucking developed abnormal oral behaviors such as milk sucking in adulthood (Keil et al., 2001). Self-sucking and milk stealing is thought to be one of the reasons dairy producers hesitate putting heifers in groups (Spinka, 1992; Keil et al., 2000). Decreased milk yield from self-sucking and inter-sucking may cause udder malformations and potentially increased risk of mastitis (Keil et al., 2000). Calves exhibiting such behaviors may be culled before getting into the dairy herd and cows are generally culled if they continue to express abnormal oral behaviors. Individually housed calves may still express self-sucking which could be from the lack of social contact and objects in the pen. Veal calves deprived of objects in their pen spend more time licking their housing (e.g. feed grille and partition) as well as licking their lips and tongue rolling when compared to calves provided a tire or chain to nibble on (Veissier et al., 1997).

The motivation to suckle is a natural reaction of the beef and/or dairy calf to find nourishment from its mother. Incidences of abnormal oral behaviors give indication to a potentially compromised welfare of the animal performing these behaviors. Lack of the ability to

perform natural behaviors is a primary concern of animal production that can lead to unwanted or detrimental oral behaviors in calves. The majority of abnormal oral behaviors are associated with the welfare of dairy calves in an isolated environment. Most dairy herd's house calves individually in the United States: 67.9% used individual pens or hutches and 8.9% in tie-stalls (USDA, 2008). Calves in dairy production lack the opportunity to nurse their dams and are strictly fed from a bottle or bucket. Young ruminants who are removed from their dams perform more non-nutritive sucking on pen mates, parts of housing system, and nonfunctional teats even after receiving nourishment (Passillé et al., 1992; Hafez and Lineweaver, 1968; Wood et al., 1967). Calves that suckle their dams for the first 4 days of life are reported to rarely suck on other calves (Krohn et al, 1999), but cross-sucking and inter-sucking may still occur after weaning off the cow (Keil et al., 2000). Although non-nutritive sucking looks to have no function the behavior may allow for satiation in calves, leading to reduced motivation to suck (Passillé and Rushen, 1992). Non-nutritive sucking may be important to the natural behavior of the calf even if provided plenty of nutrition through milk and diet after weaning. Calves are stimulated to suck by the ingestion of milk and occurs the most within 10 min following a meal (Passillé and Rushen, 1997). Non-nutritive oral behaviors do not cease even if the calf receives adequate nutrition meaning this behavior is part of its natural behavior (Passillé, 2001).

Enrichment of a dairy calf's living environment may benefit the calf's welfare and aid in the reduction of unwanted behaviors. Providing a dry teat to dairy calves after a meal reduces non-nutritive sucking on pen mates and other parts of the calf's pen (Rushen and Passillé, 1995). The public concern for animal welfare is related to the health of the animal, amount of pain or aversive emotions the animal suffers from, and the ability of the animal to perform normal behaviors (Fraser et al., 1997). These normal behaviors are basic to all animals which could

include lying, standing, eating, drinking, grooming, and others. Expression or the deprivation of these behaviors could shine light on how well animals are coping with a production system. Grouping animals may lead to better perception of animal welfare but bring in other welfare issues including unwanted social behaviors (i.e. cross-sucking and agonistic interactions) and sickness (Rushen, 1994). In a group setting tying up of a calf for 10 min after a milk meal was shown to reduce the incidence of cross-sucking (Graf et al., 1989) but this method does not give the calf opportunity to perform non-nutritive sucking.

### **Phylogeny**

Domestication and various production systems can affect calf oral behaviors in numerous ways. The major differences in production systems can be seen with beef cattle and dairy cattle. Beef cattle are typically raised in extensive production systems; they have less human intervention/input when compared to dairy cattle production. Beef calves are generally handled very little after birth and allowed to remain with their dam. In a national sample survey conducted from December 1996 to February 1997, heifers and cows were checked an average of 3.6 and 2.5 times per 24-hour period during the calving season (Dargatz et al., 2004). In addition, 39.6% of calving's took place in a calving area, which is a small number when compared to dairy cows. In contrast, dairy calf removal from the dam immediately after birth is common practice and monitoring of the cow and calf happens numerous times throughout a 24-hour period. In a study conducted by the NAHMS of 1,623 dairy heifers, 63.2% were bottle fed, 10.3% esophageal fed, 2.0% both bottle and esophageal, 0.2% bucket fed, and 24.2% suckled from their dam (Shivley et al., 2018). This practice of removing the calf immediately is to reduce the risk of the calf being exposed to environmental pathogens (Windsor and Whittington, 2010). Johne's disease in ruminants results in severe diarrhea in infected animals and consequently

death. Infected animals shed bacteria, *Mycobacterium paratuberculosis*, in feces and milk. Calves nursing dams with Johne's disease can be infected resulting in culling or death of the calf. In a study conducted by the National Animal Health Monitoring System (NAHMS) found 68% of U.S. dairy herds had at least one cow that tested positive for Johne's disease and a near 100% prevalence in large dairy herds. The occurrence of Johne's disease resulted in a push for farmers to reduce nursing of the dam by the calf to reduce transmission of the disease. Producers removing calves immediately after birth (no nursing of the dam) increased 28.0 to 47.9% from 1991-1996 (USDA, 2008).

Increased handling and close animal-human relationships in dairy production may have decreased the level of precociousness in neonate dairy calves. Murray and others popularized the term "calf vigor," however; this term is limited to the calf immediately after birth. Therefore, the term, precociousness encompasses the entire development of the calf. Dairy calves are less precocious after birth (e.g. dairy calves take longer to stand) compared to beef calves which could lead to morbidity and/or mortality issues. There is evidence that the greater the latency of calves to obtain its first meal, the less protective colostrum ingestion is for the calf. Therefore, both beef and dairy calf raisers need to monitor calves to insure adequate amounts of colostrum (10-12% of body weight) are ingested within a 6-24-hour time period. Normal sized dairy calves are recommended to consume 3 L of colostrum within 4 hours and a total of 4 L within 12 hours from birth (Chigerwe et al., 2009). Beef calves are more precocious than dairy calves and take less time to stand and nurse. Regardless, both dairy and beef calves that do not stand and suckle within 6 hours after birth have a higher risk for failure of passive transfer of immunity (Besser et al., 1991; Edwards, 1982).

### **Applied Ethology and types of cattle management systems**

The challenge with understanding behaviors in bovine calves originates from the vastly different raising systems for different calves. Dairy calves are raised in an intensive production system, while beef calves are raised in a more extensive production system. The primary difference between the two is how much handling and care the calf receives after birth. In the dairy industry calves are monitored daily and readily easy to handle. This allows for easier collection of behavioral and performance data compared to beef calves. Furthermore, less vigorous/precocious calves such as dairy calves make treating animals easier for producers. The majority of dairy calves are born in a maternity pen giving increased access to the calf and less labor involved in monitoring cows in labor. In addition, the lack of maternal bonding in dairy calves gives rise to trying to better understand how this affects calves after birth and early in life.

Beef calves are more precocious than dairy calves and are raised in thousands of acres of land. For example, the average ranch in South Dakota is 1,397 acres (USDA, 2017). Nonetheless, extensive cattle production systems are challenging for data collection of beef calves. Beef calves may not be monitored until 12 hours or more after birth due to the decreased supervision of the producer in that setting. In those 12 hours calves may not receive adequate colostrum from suckling, increasing the risk of morbidity and mortality. Increased understanding on suckling of the udder by beef calves could help determine if passive transfer of immunity is adequate in the first hours of life. In comparison, dairy calves are born into a very intensive system, and are provided a lot of human support at birth. Calves born in an intensive setting are likely to receive adequate amounts of colostrum if management is being done to ensure passive transfer of immunity. However, calves are hand raised in individual housing and thousands can be raised on a few hundred acres of land by just a few calf raisers. These systems were optimized

for efficiency, but not for meeting a calf's natural desire to express suckling. Therefore, this thesis uses applied ethology techniques to address the needs of both beef and dairy calves.

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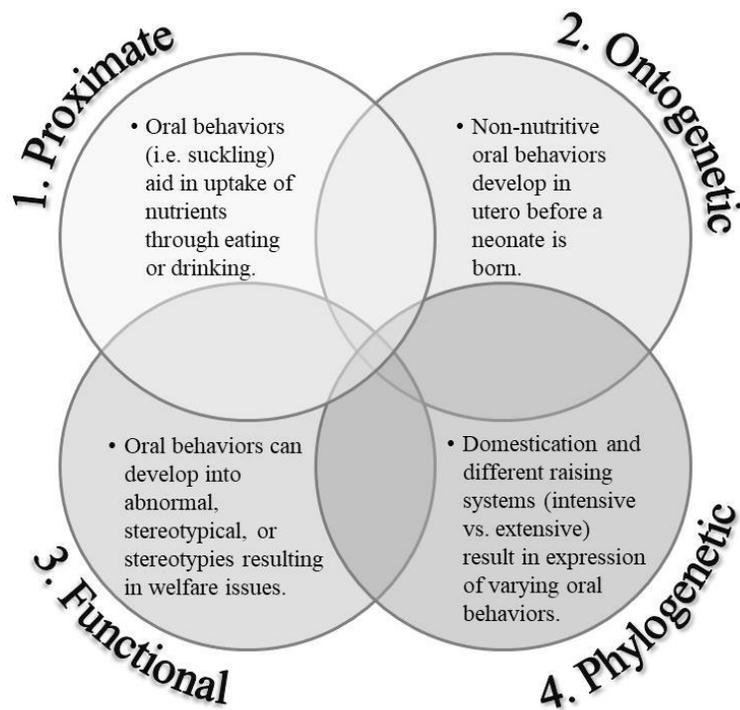
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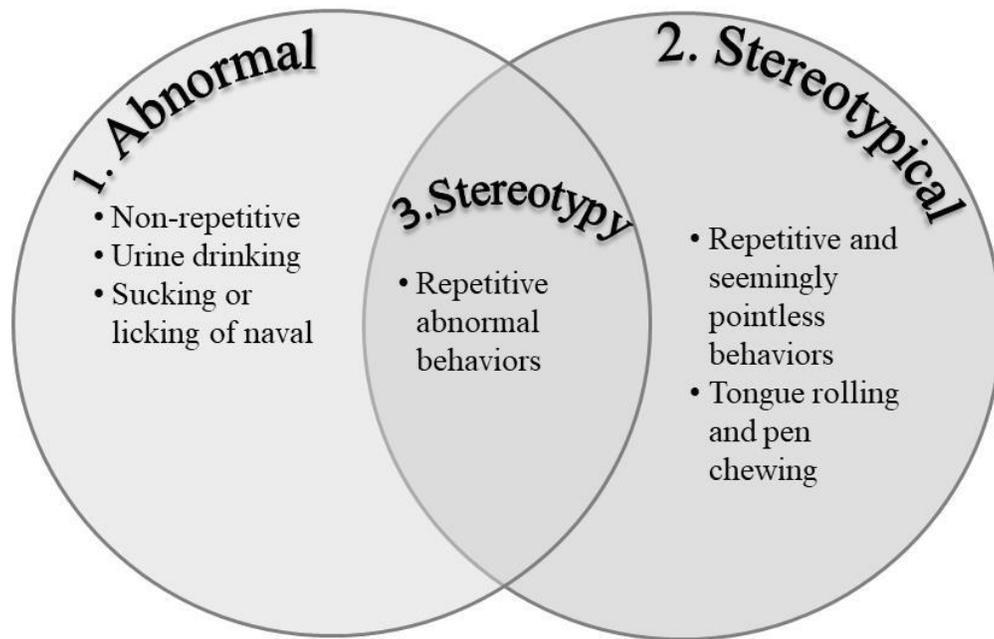
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**Figure 1-1.** Oral behaviors can be organized into four behavioral categories: 1. Proximate, 2. Ontogenetic, 3. Functional, and 4. Phylogenetic. Behaviors can be associated with multiple categories and explanation of a certain behavior involves taking into account all other categories.



**Figure 1-2.** Oral behaviors based on a functional standpoint can fall into these three categories if they are not normal- abnormal, stereotypical, and stereotypy. Categorizing behaviors aids in management of calves and better understanding behavioral needs. Adapted from Mills, D. S., and J. N. Marchant-Forde. (Eds.). 2010. The encyclopedia of applied animal behaviour and welfare. CABI. Page 521.

# **Chapter 2 - Automated and Visual Data Collection Techniques for Dairy Calves.**

## **Abstract**

Automated data collection of animal behavior is expanding knowledge in all aspects of animal care. Increased understanding of what animal's need is of primary focus in collecting of behavioral data. The objective of this validation experiment is to evaluate and validate an environmental enrichment device (EED) and provide insight to how behavioral data is collected using video recording systems and automated devices. This experiment was part of a larger project conducted in the summer of 2014 at Texas Tech University. Holstein bull calves (n=6) were fitted with 3-axis accelerometers at 5 d of age and provided environmental enrichment device from d 5-68 of age. A total of 59 h of video footage was analyzed for calf behaviors. Observed EED use was shown to be highly correlated with automated EED ( $r=0.98$ ;  $P<0.0001$ ). Observed standing and lying durations were correlated with automated standing and lying durations (standing-  $r=0.97$ ;  $P<0.0001$  and lying- $r=0.97$ ;  $P<0.0001$ ). Use of environmental enrichment can aid in collection of data and allow animals to express natural behaviors such as suckling. Furthermore, data collected through automated techniques can give valuable knowledge without increasing distress to animals and providing more time efficient methods of data collection.

## **Introduction**

The use of automated data collection of behavior will improve the management of animals for food, companion, service, and research. The use of automated data from an environmental enrichment device (EED) will serve as anabolic animal welfare benefits because

the animal's natural behaviors can be expressed, the manager can monitor the behaviors, and in turn make more timely decisions to improve health and productivity of the animal. Furthermore, data potentially can be collected with minimal human interaction, this limiting distress to the animal. Some examples of technology in raising animals include radio-frequency identification (RFID), accelerometers in ear-tags/collars, and video surveillance systems. Dairy farmers are a large population of technology supporters using these tools to better understand and manage their animals. In research, use of video data collection techniques and automated activity data, gave insight to daily activity, feeding patterns, and validation of other technologies (i.e. environmental enrichment devices). The 3-axis accelerometers were used by many scientists to establish activity patterns such as standing, lying, and movement in cattle (White et al., 2008; Passillé et al., 2010; Rushen et al., 2012; Bonk et al., 2013; Calvo-Lorenzo et al., 2016). The goal of this protocol is to develop a method for collecting calf behaviors in single-housed calves, which is a very common raising strategy in dairy calf raising systems.

## **Materials and Methods**

This validation-experiment was part of a larger project (Sharon et al., 2016) that was conducted in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the Institutional Animal Care and Use Committee of Texas Tech University (IACUC # 14011-02) in the summer of 2014. For the validation experiment, 6 Holstein bull calves (1 d of age) were used. A total of 59 h of video footage was analyzed for calf behaviors (Table 1). All calves were fed twice daily, 0730 and 1630 h, with commercially available milk replacers (Herd Maker, Land O'Lakes Animal Milk Protein Co., Shoreview, MN or Cow's Match, Land O'Lakes Animal Milk Protein Co., Shoreview; see

Sharon et al. for nutrition details) and were offered ad libitum access to a texturized calf starter and water.

At age 5 d, accelerometers (UA-004-64; Onset Computer Corp., Bourne, MA) were fastened to the calf's right or left hind leg with Vetwrap cohesive bandage (3M Products, St. Paul, MN). A custom-designed dummy-nipple with a sensor (Environmental enrichment device-“EED”); described by Hulbert et al., 2015; created by Meter Mall USA, Marysville, OH) was left in the bottle-holder of the hutch for d 5-68 of age, except for during milk-replacer feedings. The sensor is located in the dummy nipple, and when the calf manipulated the nipple (suck, head-butt, push, etc.), the sensor (SW-18020P, ADAFruit, New York City, NY) activated the attached event-recording logger (UX90-001M1; Onset Computer Corp, Bourne, MA). At midnight at age d 6, the accelerometers and event loggers began recording every 30 s and each time the calf manipulated the dummy-nipple (at a rate of 1 Hz). Every 1-3 wk, all logging devices were removed, data were downloaded and processed using methods previously described (Ledgerwood et al., 2010; Hulbert et al., 2015); standing duration and environmental enrichment device (EED) use (min/day) were the main variables of interest.

### **Step 1. Setting up pens for individually housed calves outdoors**

- A. Prior to experiment, set three calf hutches and pens within 30 cm of each other.
  - a. **Note:** This spacing allows for one outdoor camera to capture 3 pens.
- B. Place two buckets, one for solid feed and one for water, as well as a bottle holder.
  - a. Provide ad libitum starter and water. Change water and check for soiled feed at least once daily.
  - b. Fresh bedding (sand in the summer, straw or other substrate in the winter) should be provided and checked routinely for soiling.

- c. A bottle of milk replacer is available during the morning and evening feeding times. All other times a custom-designed dummy nipple (Figure 1) is placed in bottle holder.

## **Step 2. Setting up pens for individually housed calves indoors**

- A. The barn is setup with 4 rooms that accommodate 120 calves per room.
- B. Individual stalls are set in a manner that utilizes the most space in the calf barn.
- C. Stalls are 2.13 m x 0.61 m constructed from wood and Tenderfoot flooring (Tandem Products, Inc., Minneapolis, MN).
  - a. Check pens regularly for build of fecal material and clean when necessary.
- D. Each stall has a removable plastic divider to allow pair housing with adjacent calf around 9 weeks of age.
- E. Buckets for milk and starter should be placed at the front of each pen.
  - a. Provide water and starter ad libitum.
  - b. Milk is available in two feedings, morning and evening.

## **Step 3. Setting up the video recording system for outdoor housed calves**

- A. Prior to placing animals in their pens, cut a 7 cm diameter hole into each of the plastic hutch roofs for camera installation.
  - a. Place one camera with a wide-angle lens at a 90° angle in the top of each of the three calf hutches using the hole cut in previous step at 1.27 m from the floor. Place a fourth camera at 90° and 2.13 m above the hutches to capture behaviors in the outside pen area.

- B. Using Ethernet cables connect the cameras to a single switchboard (TPE-224WS, Trendnet, Inc., Torrance, CA.) that is connected to computer monitor and tower being used for the study (NVR-Rack64, Points North Surveillance Inc., Auburn, ME).
  - a. The recording system can be stored inside a heavy-duty toolbox in an extra hutch centrally located in the study area.
- C. Video cameras (Bullet IP Camera, GV-EBL2111, Geovision Inc, Taiwan) record footage continuously in HD 1080p quality onto four--4 TB drives.
  - a. The 4 TB drives record footage for all cameras throughout the study without the need for downloading data and clearing the drives.
  - b. **Note:** Video recording system should start at midnight to ensure more accurate time keeping. Timestamps added to videos do not accurately represent time when lining up data from automated and observed data. Recordings starting at midnight can more accurately track time by using a frame per frame basis.

#### **Step 4. Setting up the video recording system for indoor housed calves**

- A. Attach one camera with wide angle lens to the ceiling of the barn at a 90° angle at 3.35 m from the ground.
  - a. Camera should be situated in the middle of two stalls to adequately record video across four stalls.
- B. Install Ethernet cables and run the cables to a central location where video recording system is placed.
  - a. Be sure to manage extra cable with zip ties or Velcro cable wraps to avoid accidental tripping or pulling of cameras.

C. Connect Ethernet cables to switchboard that is connected to computer and tower being used for the study (NVR-Rack64, Points North Surveillance Inc., Auburn, ME).

- c. **Note:** Video recording system should start at midnight to ensure more accurate time keeping. Timestamps added to videos do not accurately represent time when lining up data from automated and observed data. Recordings starting at midnight can more accurately track time by using a frame per frame basis.

### **Step 5. Preparing and fastening the accelerometers for standing and lying behaviors**

- A. Launch the loggers by placing the logger (UA-004-64; Onset Computer Corp., Bourne, MA) into the shuttle.
- B. Open the Hoboware software on computer/laptop.
- C. Select “Device” and click “Launch.” Click OK when prompted.
- D. Make sure the logger is properly named. The calf number **MUST** be the last numbers of the file name.
- E. Under “Channels to Log,” Select Z-axis Acceleration and Y-axis Acceleration only.
- F. Under “Logging Interval,” select Normal and put in 1 minute.
- G. Select date and time to launch logger (midnight d 6 of age).
- a. **Note:** Launching loggers at midnight is crucial for data to be aligned with video data. Launch the loggers from the same computer being used for the video recording to ensure times are exact for both video recording and automated devices.
- H. Click “launch” and record launch start time and date on log sheet.

### **Step 6. Prepare fastening materials for logger**

- A. Wrap each launched logger in plastic wrap or cellophane to prevent excessive water damage.
- B. Use marker to write identification on the plastic wrap or piece of tape.
  - a. If using tape to apply identification number be sure logger is oriented the correctly on the calf.
- C. Cut one 6.35 x 20.3 cm strips of non-slip shelf liner for each calf. Cut this strip in half (two- 10.15 cm long pieces; one for the outside of the leg and one for the inside).
- D. Apply a 3.8 cm strip of Velcro to the middle of the non-slip liner (this will go on the inside of the leg to hold the logger).
- E. Apply the matching side of the Velcro to the back of the logger (the side of the logger that is clear in color not purple).

#### **Step 7. Restraining calf**

- A. One person gently place the calf on its side (right or left) depending on side chosen for rest logger.
  - a. **Note:** The calf's back should be facing the person who placed it on its side, while on the ground.
- B. Place one knee on the flank or hip of the calf and the other knee on the shoulder of the calf.
  - a. **Note:** The person holding calf should put enough pressure to keep the calf from getting up.
- C. Hold the calf's front legs down with your hands to prevent kicking as well as the hind leg not receiving the logger.
- D. A second person should be on the belly side of the calf to place logger.

## Step 8. Fastening the logger to the calf

- A. Smear a 10 cm thin layer of tag cement (Nasco, Modesto, CA) onto the non-slip liner without the Velcro attached. Add this 10.15 cm liner to the lateral side (outside) of the hind leg of choice just below the hock joint (Figure 2a). Wrap the leg and liner with 2 layers of Vetwrap (3M Products, St. Paul, MN) and check for tightness using two fingers (Figure 2b).
- B. Apply a small amount of tag cement to the back of the shelf liner with the Velcro.
- C. Place the non-slip liner with Velcro on the medial side (inside) of the leg on top of the Vetwrap (Figure 3a-b).
- D. The logger is put in place by attaching the Velcro of the logger to the Velcro of the non-slip liner. Make sure the logger is oriented with the purple side facing out and the y-axis of the logger aligned with the leg (Figure 4a-b).
- E. Apply 3-4 more layers of VetWrap around the leg making sure to cover the logger and non-slip liner. Cut the VetWrap and secure the end piece to itself on the inside of the leg. Check the tightness of the VetWrap using fingers—should be able to get 1-2 fingers between VetWrap and calf's leg easily (Figure 5).
  - a. **Note:** It is crucial the VetWrap is just tight enough to prevent the logger from slipping or falling off. If the VetWrap is too tight it may restrict blood flow to foot of the animal and cause increased irritation/stress to the animal when trying to remove the logger. Additionally, the use of other wrap types and brands (e.g. Coflex) should be used with caution. Similar types of wraps may actually shrink resulting in a wrap that causes increased constriction to the limb. The restriction of blood flow to the limb results in discomfort and potential mobility problems.

### **Step 9. Removing loggers from calf**

- A. Restrain calf gently while leaving hind leg with logger attached accessible.
- B. Using blunt-ended scissors, cut through VetWrap on the outside of the leg from top to bottom without puncturing the calf or logger.
- C. The non-slip liner on the lateral side of the calf's leg can remain on the calf and will fall off with time.
  - a. This reduces irritation to the leg by not tearing the liner off and causes the calf no increased stress by leaving the liner on. The natural processes of skin death, hair shedding, and daily calf behavior (e.g. scratching, rubbing, lying and standing) will allow the liner to fall off more naturally.
- D. Detach the logger from the non-slip liner by separating the Velcro. Store the logger in a container for later handling.
- E. Record the time of removal from the calf on a datasheet.

### **Step 10. Downloading data**

- A. Place the logger into the shuttle.
- B. Open the Hoboware software on a computer/laptop.
- C. Select "Device" and click "readout." Click OK when prompted to on the computer.
- D. The file should be saved in a folder labeled "Hoboware Calf Data" in My Documents.
- E. Click "plot" when prompted. Select "file" and click on "export table data." Make sure "export as a single file" is selected and click "export."
- F. Save the file in My Documents in the folder labeled "Excel Calf Data."
- G. Repeat this for all the loggers used.

## Step 11. Preparing the ethograms for video data

A. Structural behaviors are associated with the whole body or parts of the body of the calf.

This calf study had contained three structural categories: 1) calf's location in its pen, 2) position of calf, and; 3) oral behaviors of the calf (Table 1).

- a. The calf's location in the pen is categorized by "in" or "out." The calf was considered to be "in" when the head of the calf was inside the hutch (Figure 9A). The calf was considered "out" when the head was located on the outside of the hutch in the pen area (Figure 9B).
- b. Oral behaviors are categorized in six mutually exclusive states.
  - i. Head-still- The head of the calf is at rest, not sniffing, licking or performing any other oral behaviors (Figure 10C).
  - ii. Non-nutritive oral behaviors (NNOB) - any non-nutritive oral behavior: licking, biting, or sniffing an object, grooming, head-butting or sucking objects other than the EED (Figure 11A).
  - iii. Environmental enrichment device (EED) - The calf's mouth makes contact with the EED dummy nipple (Figure 11C).
  - iv. Feed- The calf's head is located in the feed bucket (Figure 11D).
  - v. Water- The calf's head is located in the water bucket (Figure 10D).
  - vi. Milk- The milk bottle is present and the calf is suckling milk (Figure 11B).
- c. The calf's position is categorized as "stand" or "lying." The calf was considered "standing" when an upright position was maintained with at least 2 hooves (front

or back) were in contact with the ground (Figure 10A). Calf is considered “lying” while on the ground in a recumbent position (Figure 10B).

## **Step 12. Preparing of video data and timestamping**

- A. Video data for original analog camera system is edited and rendered using Corel Studios Software (VideoStudio Pro X8, Corel, Ottawa, Canada).
  - a. Click “Edit” in the top taskbar if not already selected after opening program.
  - b. In the pane to the right of the video screen select “Add” to add a new folder for video data and name based on study.
  - c. Select the folder icon to the right of the pane where the folder was just added.
  - d. In the pop up display find and select files needing to be rendered.
    - i. The analog camera system saved video data in five minute clips.
    - ii. Find and select all clips needing to be put together for each observation or calf.
  - e. Files will be uploaded in the area just below the folder icon.
  - f. Drag and drop each file in order, based on time, in the first row of the timeline just below the video screen.
    - i. Do not leave gaps in the timeline. Each video clip should be right next to the previous with no space in between.
  - g. Audio can be removed from video if present and desired.
  - h. After inserting all appropriate video clips, select “Share” in the top task bar.

- i. Select the appropriate format of video (e.g. AVI).
  - j. Name the file in “file name” and select a place to store in “file location.”
    - i. The file name should include the identification of calf, date/time, study name, and other distinguishing information.
  - k. Click “Start” to render the video.
- B. Video data for the newer and recommended system (Geovision, Points North Surveillance Inc., Auburn, ME) is equipped with rendering software.
- a. Open Geovision software.
  - b. Click the icon with the magnifying glass in the bottom right hand corner of the screen.
  - c. Select “Video/Audio Log (F10)” from the options provided.
  - d. Select the camera of choice from the dropdown in the upper-right hand corner of the video screen.
  - e. Select the date containing video footage needing to be rendered.
  - f. In the “video events” pane select the range of time the video of choice is located.
    - i. The range should be highlighted gray.
  - g. Select “Save as AVI” icon on the right side of the screen.
  - h. Input the start time and end time in the space provided for the video needing to be rendered.
  - i. Select the “setting” tab in the upper-left corner.

- j. Set a location for the video to be saved by clicking the button containing “.....” in the upper right corner.
      - k. In “general setting” select standard merge.
      - l. In “codec selection” select WMV9.
      - m. Click “OK” to begin rendering process.
- C. Trained observers can be used to timestamp videos; if two or more trained observers are used, intra-observer variation should be calculated.
  - a. Select “Reliability Analyses” then “New Reliability Analysis” in Project Explorer pane.
  - b. Select an observation in “Observations” and click “Add.”
  - c. Select a second observation in “Observations” to compare to first observation and click “Add”.
  - d. Both observations should appear in the same line (e.g. 1, 2, 3, etc.) in the “Observation Pairs” box.
  - e. Click “OK” and the software calculates the percentage of agreement between the two observations.
- D. Ethograms are constructed under one project using special software (The Observer XT 11.5; Noldus Information Technology, Leesburg, VA).
- E. Playback speed should remain at 1X for position and location behaviors (i.e. 30 frames per second). The oral behaviors may be slowed to .5X (i.e. 15 frames per second) speed to better distinguished behaviors being performed.
- F. Timestamp each category of behaviors separately.
  - a. Start by timestamping location and position behaviors simultaneously.

- b. Restart the video after completing these two categories.
  - c. Next, timestamp the oral behaviors.
- G. The timestamping of videos needs to have standards set by each research group and all observers need to be trained with the same methods.

### **Step 13. Data from observer**

- A. From this timestamping, the duration and frequency of each behavior can be used.
- B. Select “Data Profile” under analyses in Project Explorer pane.
  - a. Make sure a “Results” component is present in the profile.
- C. Select “Behavior Analyses” then “New Behavior Analyses” under analyses in Project Explorer pane.
- D. Select “Statistics” in upper right corner of Behavior Analyses window.
- E. Check the boxes for “Total Duration” and “Total Number” and select “OK.”
- F. Select “View Settings” then “Observations” in upper right corner of Behavior Analyses window.
- G. Check the boxes for all observations being exported and click “OK.”
- H. Select “Calculate” in upper left-hand corner of Behavior Analyses window.
- I. Select “Export” in upper-right hand corner of Behavior Analyses window.
- J. Name file and pick location to be saved and click “Export.”
- K. The specialized timestamping software can export all data needed into a single Excel file.

### **Results**

Data was analyzed for descriptive statistics and Pearson's correlations using SAS (version 9.4, 100 SAS Campus Drive, Cary, NC). Twenty behavioral bouts of automated and observed EED were summed and analyzed for Pearson's correlations. Observed EED use was shown to be highly correlated with automated EED ( $r=0.98$ ;  $P<0.0001$ ). Observed standing and lying durations were correlated with automated standing and lying durations (standing-  $r=0.97$ ;  $P<0.0001$  and lying- $r=0.97$ ;  $P<0.0001$ ). The ethogram presented for the time period sampled was reliable in observing all behaviors represented (Table 1). Milk was left out of the descriptive statistics due to minimal occurrence and ability to observe time milk bottle was placed. Data analyzed using The Observer XT produced an inter-observer reliability  $>95\%$ .

Observed EED had a high variation from video sampled (CV= 88 % Pre-weaning and 176.4% Post-weaning; Table 2). Variation was reduced using automated data for animals in 24-hour periods but still present (CV= 50.1% Pre-weaning and 169.3% Post-weaning; Table 2). Differences in variation stem from some calves using the EED a great amount of time while others use it very little. Sharon and others (2018) showed EED was able to detect treatment x time differences ( $P=0.013$ ) in high vs. low plane of nutrition calves while rest loggers did not detect a difference. Power-calculations show reduced replications needed for automated data when looking at automated EED use compared with observed EED use (e.g. 15% difference Pre-wean: observed-724, automated-235; Table 2).

Individually housed outdoor calves spent 38.25% (SD 5.4%) of their time standing before weaning during the sampled video times. Calves spent 28.71% (SD 9.4%) of their time standing after weaning during the sampled video times. Automated standing data showed pre-wean calves stood 26.33% (SD 3.4%) on average during a 24-h period. Post-wean calves stood 26.97% (SD 4.1%) on average during a 24-h period. During the sampled video pre-wean calves performed

non-nutritive oral behaviors 38.0% (SD 15.9%) of the observation time and post-wean calves 23.4% (SD 4.7%).

Calf management systems (i.e. indoor and outdoor) result in differences in lying and standing patterns in calves. Figures 12A and 12B represent standing and lying durations for a calf raised in an indoor setting. Figure 13A and 13B represents standing and lying durations for a calf raised in an outdoor setting. The use of the EED can also be different for varying management systems. Figure 12C represents the use of the EED by a calf in an indoor setting while figure 13C represents a calf raised in an outdoor setting. The EED may help show differences in calf behavior or health more easily than the rest logger due to the EED being used primarily around feeding times. The EED allows for better determining of sample times because behaviors such as oral behaviors occur primarily around feeding periods. Figures provided are examples of data that can be collected using the toolsets outlined in this study.

## **Discussion**

### ***Video Camera Quality***

The data collected for the represented individually housed calves initially used an older analog camera type (Panasonic WV-CP310, Newark, NJ) with 1.4 varifocal lenses (Pelco, Clovis, NM USA) which is budget friendly and durable, but lack in the quality of the image being recorded. Analog cameras needed to be setup in their correct position (top of hutch and/or on pole for pen data collection) and then manually focused to ensure the calves were visible. Video cameras not focused properly recorded blurry data, which could not be used for timestamping detailed behaviors such as non-nutritive oral behaviors.

Analog cameras utilized previously need to have additional red lights installed allowing for infrared imaging of calves during the night. The use of these lights can intrigue curious

calves resulting in additional non-nutritive oral behaviors towards the string lights. Placement of red lights should be in a way that causes little interaction with the calf. Adequate string lights need to provide additional illumination to outside pen and inside calf hutch.

The recommended video camera system provided by Points North Surveillance, records high definition 1080p footage continuously and is a single unit (lens and camera). Cameras do not need to have lenses attached before mounting and have the capability to autofocus compared to the analog cameras having to be manually focused. High definition videos allow for more detailed timestamping of behaviors such as calf oral behaviors. In addition, these cameras need no additional lighting for nighttime record. The cameras utilize integrated infrared lights to record high quality video at night. Infrared properties of the camera reduce disturbance to the animals when illumination is not present naturally (sun down or lights turned off).

### ***Storage Capabilities***

The analog camera system used a four-channel recording system (DVR3-240 ST9373 PAL/NTSC Digital Video & Data Recorders, Stack, Wales, UK) and SanDisk Extreme Pro (Western Digital Technologies, Inc., Milpitas, CA) flashcards. A huge benefit of the stack DVRs is that they are very robust and can be implemented in extreme conditions. Each DVR connects to four cameras and has the capability to also attach audio recording devices such as microphones. In addition, Stack DVRs are small in size taking up less space in the environment they are deployed in. The major downside with the Stack DVRs is that they could only record for 24 hours before needing to be stopped and data downloaded or full flashcards replaced with new ones.

The newer proposed video recording system uses 4TB drives and records numerous cameras at the same time. This system can record animals continuously for multiple days without

needing new storage put in place. However, this means the newer system takes up more space and will need adequate room to store computer running the system. Although more space is required the system will still only need one additional hut in order to record all calves on a study.

### ***Behavior of Calves***

Calf “in” and “out” behaviors may show a level of play in the calves. The behaviors, bucking and stomping, occur when performing rapid in and out behaviors and are associated with play in calves. Increase in such behaviors is related to positive management and overall living environment of the calf. Play behavior is a positive behavior associated with the well-being of the calf and is reduced or absent when health and welfare is decreased (Held and Spinka, 2011). Weaning and castration practices show decreases in play behavior among calves giving indication these practices can cause distress to the animals (Rushen and de Passillé, 2012; Mintline et al., 2013). Furthermore, increases in play behavior are associate with positive events like increased milk allowance and socialization in calves (Duve et al., 2012). Play behavior can be a useful measure in determining the welfare of calves in confinement and modifications to their environment (e.g. enrichment, socialization, increased pen size, milk allowance) may help improve play behavior expression by calves.

### ***Environmental Enrichment Device and Rest Logger***

Calf use of the environmental enrichment device (EED) primarily occurs around feeding times. Increased use of EED could be in anticipation of a meal prior to receiving a milk meal or after the meal. Implications of increased EED use after a meal could be a result of decreased nutrition provided and/or motivation to suckle stimulated by milk and suckling. The act of sucking is stimulated by milk ingestion by the calf and continues after the meal (Passillé et al., 1992; 1997). Motivation to suckle, however, seems to be reduced more by the expression of

sucking behavior than by ingestion of milk (Rushen and Passillé, 1995). Calf utilization of the EED could eliminate some of the unwanted oral behaviors after a meal (e.g. cross-sucking). Abnormal oral behaviors such as cross-sucking become problematic after grouping dairy calves. Producers discourage abnormal oral behaviors to increase health and performance of calves. Adoption of environmental enrichment devices may satiate sucking motivation that would otherwise be directed at pen mates or housing. Reduction of unnecessary oral behaviors is important when animals are group housed. Oral behaviors directed at pen mates could result in lesions and culling from the herd if performance and health are affected. Milk stealing and self-sucking is suggested to be a continuation of abnormal oral behaviors established in calf-hood (Keil and Langhans, 2001). The EED could help categorize calves by the amount of oral behaviors directed towards the EED (high vs. low). Calves performing a high amount of oral behaviors could be managed differently due to the increase in cross-sucking incidences that may occur after grouping. In addition, high occurrence of EED use could be an indicator a calf may not be ready to be weaned. Gradual weaning of calves has shown to reduce the amount of cross-sucking expressed (Passillé, 2001). Furthermore, the EED may be more precise in detecting differences in calf health or performance compared to the rest logger. Differences in use of the EED, especially around feeding, would be a great indicator of abnormalities in a calf's health or performance. Standing and/or lying duration would not be as precise in detecting minor changes in health and performance due to the calf performing these behaviors throughout the day not just around feeding.

Rest logger data will vary between indoor and outdoor housed calves. The primary difference between the two housing systems is the environment. Outdoor housed calves are exposed to many types of weather conditions (e.g. rain, snow, wind, and sunlight). These

environmental factors will influence how active the calves are throughout the day. Seasonal temperature changes result in differences in lying time in dairy calves housed in a semi-opened shelter. Time spent lying down was reduced from 679.9 minutes in winter to 554.1 minutes in summer (Tripon et al., 2014). The result of varying weather conditions could be reflected in variation among calves when observing standing and lying durations from rest logger data. Indoor housed calves have a more constant living environment when compared to outdoor housed calves. Temperature is frequently being controlled with the use of ventilation. Calves in indoor living environments are not exposed to extreme conditions such as cold or heat stress. The result of a more constant environment may reflect with less variability in standing and lying duration amongst calves throughout a study. In addition, calves housed outdoors experience more stimuli from workers, machinery, and other animals. Increased presence of a human or worker could cause anticipation of a meal by the calf resulting in increased activity and use of EED.

### ***Troubleshooting Technology***

Based on previous experience timestamps placed on videos to help manage the time of the video are not accurate indicators of actual time. The best option for keeping accurate time is to insure the environmental enrichment device and rest logger are set to launch at midnight and the video recording system begins recording at midnight. Loggers should be launched utilizing the computer for the video recording system allowing a more synced time between the video data and logger data. Launching/starting of all devices at midnight allows time to be tracked using frames from video, allowing more precise lining up of data.

Data analyses revealed some discrepancies in our summarization techniques for the automated data collected. Data processed by The Observer XT and DynaSim spreadsheet did not

match up exactly resulting in misalignment of data points for automated and observed data. Misalignment of data could be a result of cutoff parameters specified by both programs not being the same. Findings allowed for modifications to be made to customized spreadsheet and progression of future behavior tracking technologies.

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**Table 2-1. Ethogram of Calf Behaviors.**

Code	Behavior	Description	Type
	<i>Position</i>		
1	Lying	Calf is on the ground in a recumbent position	State
2	Stand	Maintaining an upright position with at least 2 hooves (front or back) in contact with the ground	State
	<i>Oral behaviors</i>		
N	Head-Still	Head is at rest, not sniffing, licking or performing any other oral behaviors	State
7	NNOB	Any non-nutritive oral behavior: licking, biting, or sniffing an object, eating grass, grooming, head-butting or sucking objects other than the EED	State
P	EED	Calfs mouth makes contact with with dummy nipple	State
8	Feed	Head is in feeding bucket	State
9	Water	Head is in water bucket	State
z	Milk	Milk bottle is present and calf is suckling the milk	State
	<i>Location</i>		
c	In	The head of the calf is located inside of the hutch	State
v	Out	The head of the calf is located outside of the hutch in the pen area	State

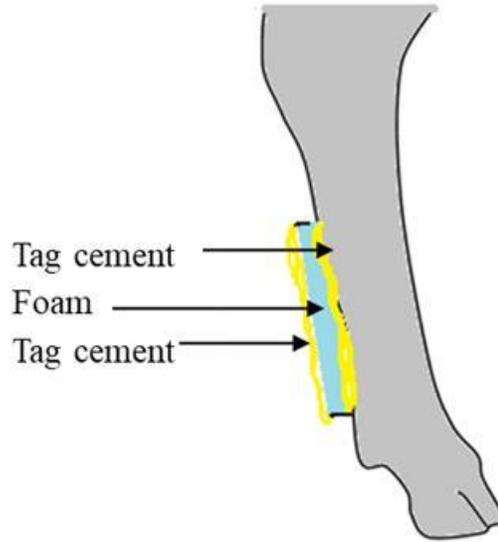
**Table 2-2. Descriptive Statistics and Power Calculations for Calves.** Video was sampled for 1200 - 1800 h for six calves before and after weaning and data are reported as % observation per 6 h period. For automated stand data, 55 d were analyzed before and after weaning and reported as % observation per 24h period. For automated environmental enrichment device data, 44 d were analyzed before weaning and 39 d were analyzed after weaning.

	Mean	SD	CV	Min	Max	Expected difference between treatment <sup>1</sup>		
						15	50	100
<b>Pre-Wean</b>								
Automated Pacifier/EED, per 24h	0.17	0.085	50.1	0.03	0.41	235	21	5
Automated Stand, per 24h	26.33	3.370	12.8	18.33	32.43	15	--	--
<i>Oral behaviors</i>								
Head-still	56.42	17.184	30.5	26.56	74.9	87	8	--
EED	0.46	0.405	88.0	0.07	1.11	724	65	16
NNOB	38.00	15.939	41.9	21.13	65.1	164	15	4
Eat	2.63	2.100	79.8	0.65	6.03	595	54	13
Drink	0.15	0.171	114.0	0	0.38	1214	109	27
<i>Spatial</i>								
In	67.45	22.783	33.8	25.68	86.73	107	10	--
Out	32.55	22.780	70.0	13.27	74.32	458	41	10
<i>Activity</i>								
Stand	38.25	5.379	14.1	32.19	45.4	19	--	--
Lie	61.75	5.379	8.7	54.59	67.8	7	--	--
<b>Post-Wean</b>								
Automated Pacifier/EED, per 24h	1.12	1.894	169.3	0.00	8.62	2678	241	60
Automated Stand, per 24h	26.97	4.118	15.3	19.38	36.25	22	--	--
<i>Oral behaviors</i>								
Head-still	66.66	7.898	11.8	55.63	75.9	13	--	--
EED	2.78	4.905	176.4	0.05	12.65	2907	262	65
NNOB	23.39	4.668	20.0	19.09	29.57	37	3	--
Eat	6.43	3.285	51.1	2.46	12.18	244	22	5
Drink	0.74	0.228	30.8	0.44	1.01	89	8	2
<i>Spatial</i>								
In	45.19	32.366	71.6	11.49	91.98	479	43	11
Out	54.81	32.366	59.1	8.02	88.51	326	29	7
<i>Activity</i>								
Stand	28.71	9.433	32.9	15.9	38.39	101	9	--
Lie	71.29	9.433	13.2	61.61	84.1	16	--	--

<sup>1</sup> Power calculations show replications needed to show specified expected differences between treatments



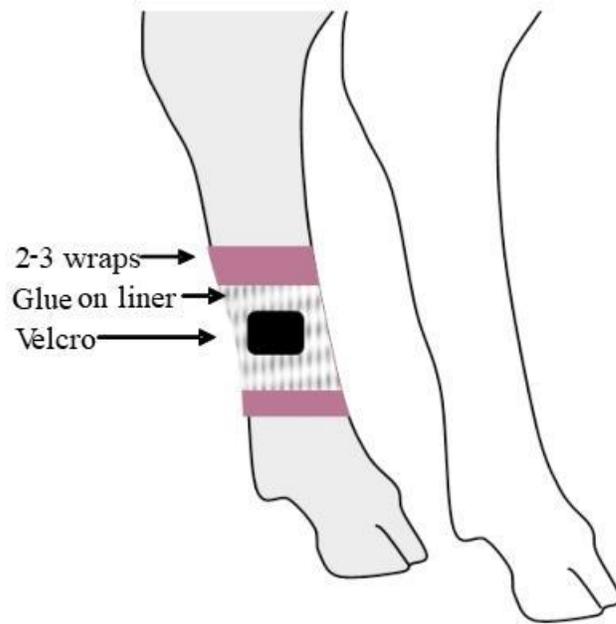
**Figure 2-1.** Environmental enrichment device (EED) contains a sensor (SW-18020P, ADAFruit, New York City, NY) housed within the bottle and dummy nipple. Calf use of the EED is automatically recorded on an event logger (UA-004-64; Onset Computer Corp., Bourne, MA) and data is downloaded periodically based on storage space, battery life, and study parameters.



**Figure 2-2.** A 6.35 x 10.15 cm strip of foam non-slip drawer liner is attached to the calf's leg with a thin layer of tag cement. Liner is then wrapped with 2 layers of Vetwrap (3M Products, St. Paul, MN).



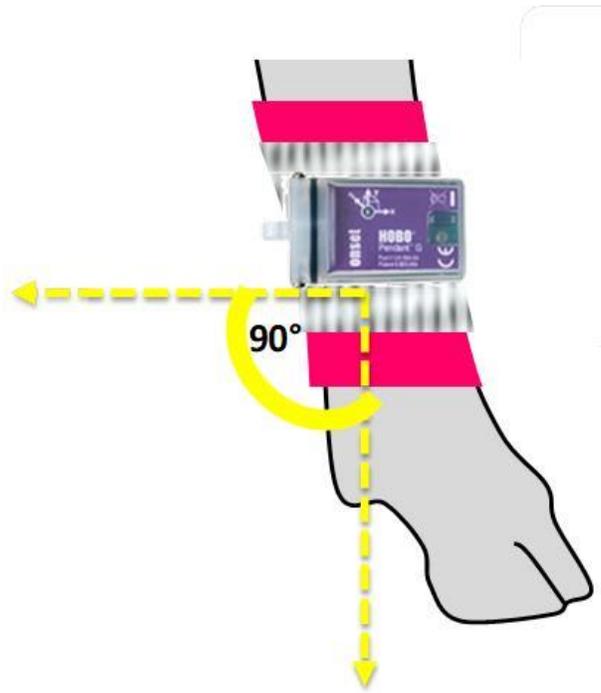
**Figure 2-3.** Wrap the leg and liner with 2 layers of Vetwrap just below the hock joint of the hind limb used to hold the logger.



**Figure 2-4.** The second piece of foam liner is attached to the inside (medial) of the leg on top of the Vetwrap with a thin layer of tag cement. Place the Velcro matching the Velcro on the logger in the middle of the foam liner.



**Figure 2-5.** The second piece of non-slip liner containing the Velcro is attached to the Vetwrap on the medial side of the leg with a thin layer of tag cement.



**Figure 2-6.** Rest logger is placed on calf's leg with purple side facing out. The y-axis of the logger lines up with the leg of the calf to insure proper orientation.



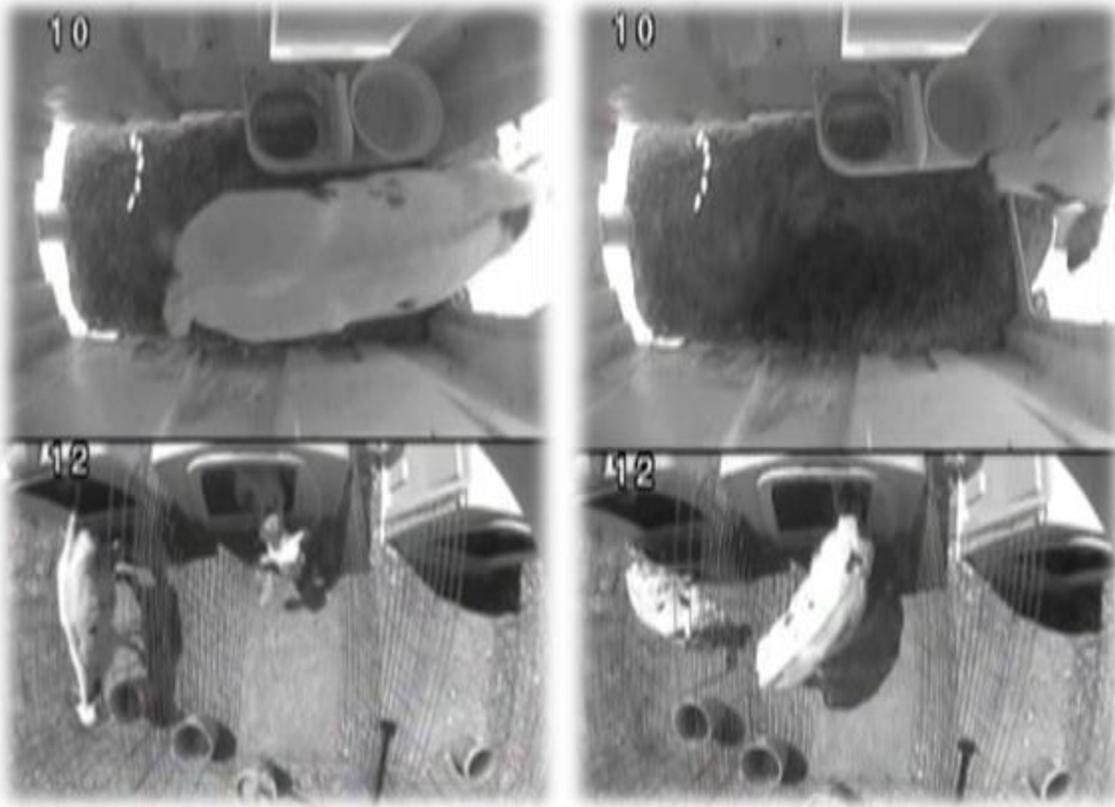
**Figure 2-7.** Accelerometer is placed with the purple side facing out (or numbered side) and the y-axis of the logger aligned with the leg.



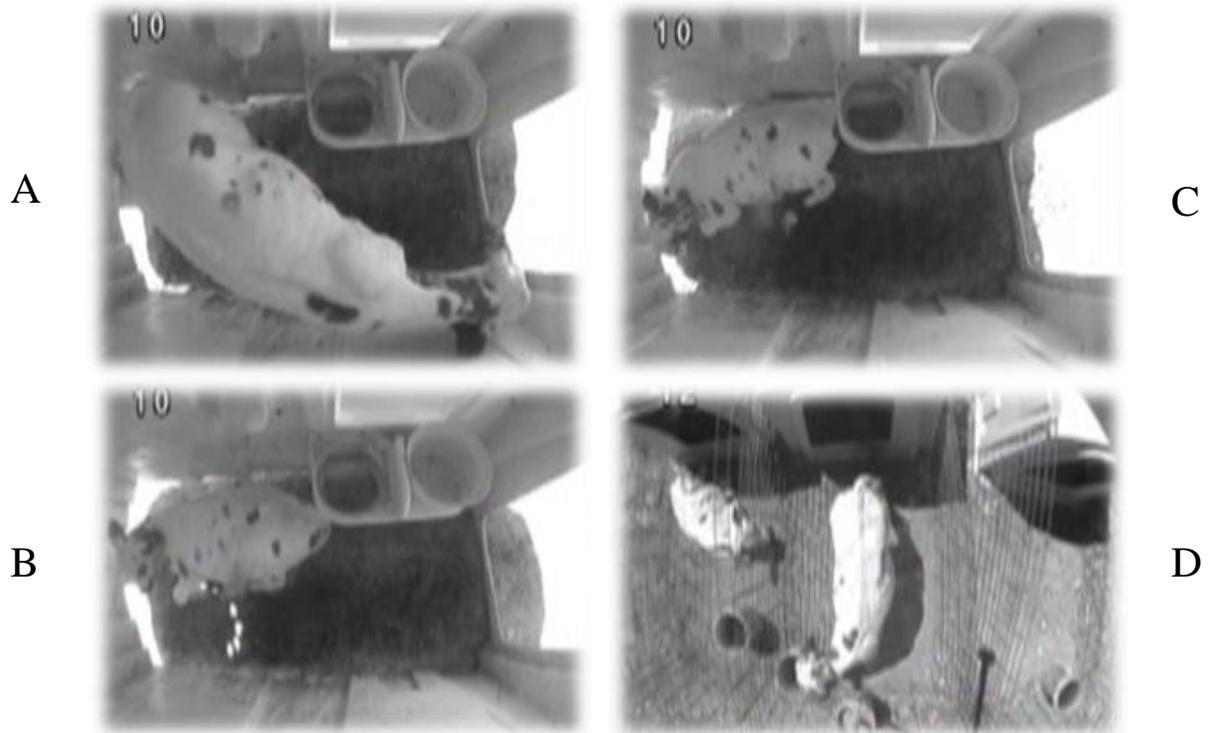
**Figure 2-8.** The 3-4 layers of Vetwrap applied around the logger should be tight enough to hold the logger in place and still be able to fit 2 fingers in between the Vetwrap and calf's limb.

A

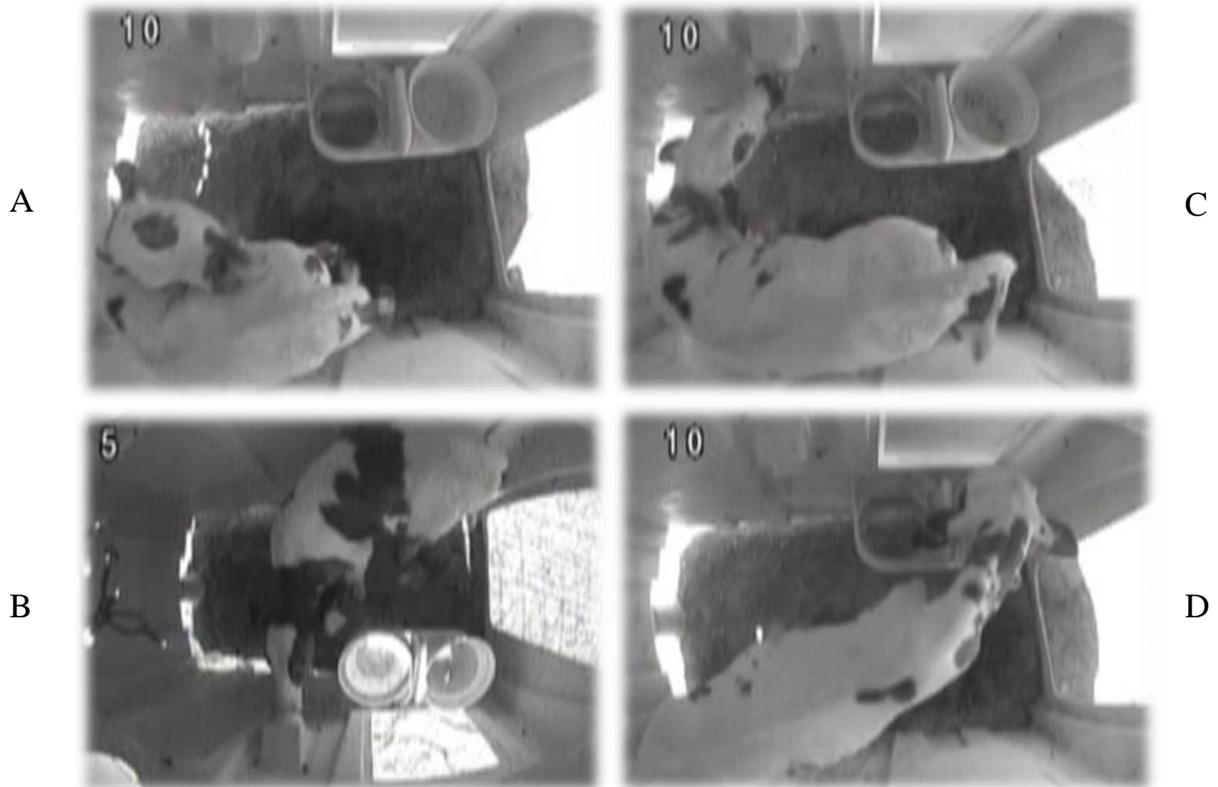
B



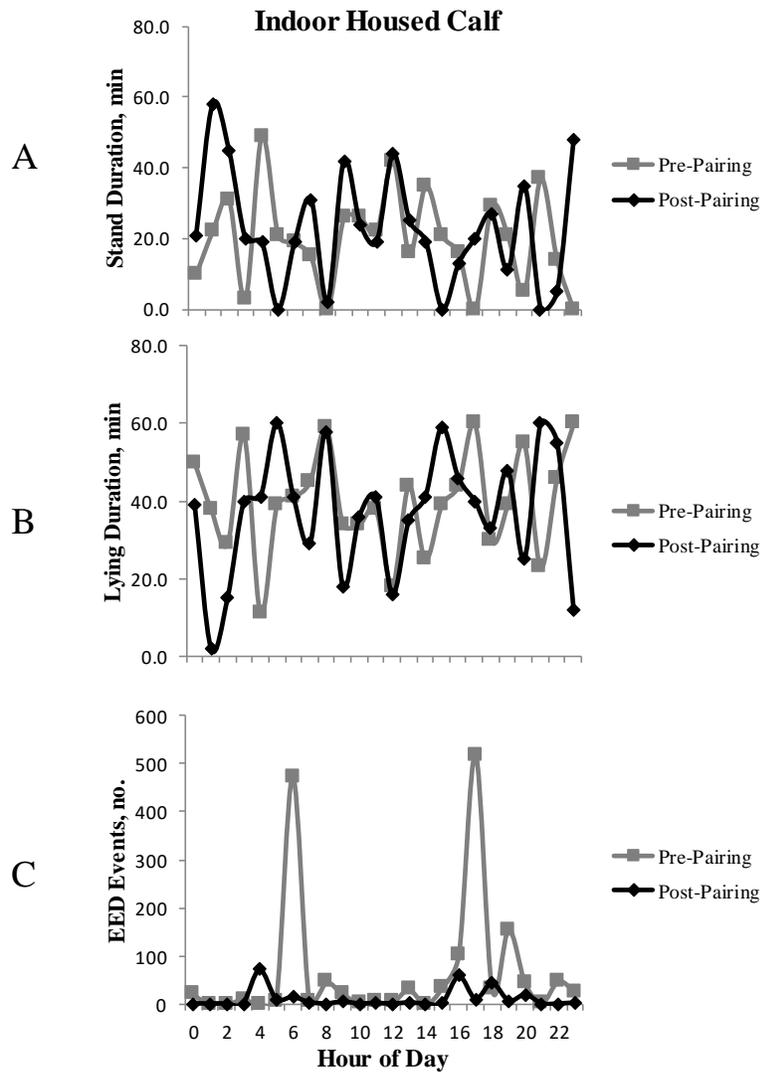
**Figure 2-9.** A. Calf is considered “in” when the head is past the opening of the hutch on the inside. B. The calf is considered “out” when the head of the calf is beyond the threshold of the hutch in the outside pen area.



**Figure 2-10.** A. The calf is considered “standing” when 2 or more hooves (front and/or back) are in direct contact with the ground and is in an upright position. B. The calf is considered “lying” when in a sternal recumbent position on the ground. C. “Head-still” is timestamped when the calf’s head is at rest, not sniffing, licking or performing other oral behaviors. D. Water drinking behavior is timestamped when the calf’s head is over or in the water bucket located in the outside pen area or if a bucket is present next to the feed inside the hutch.

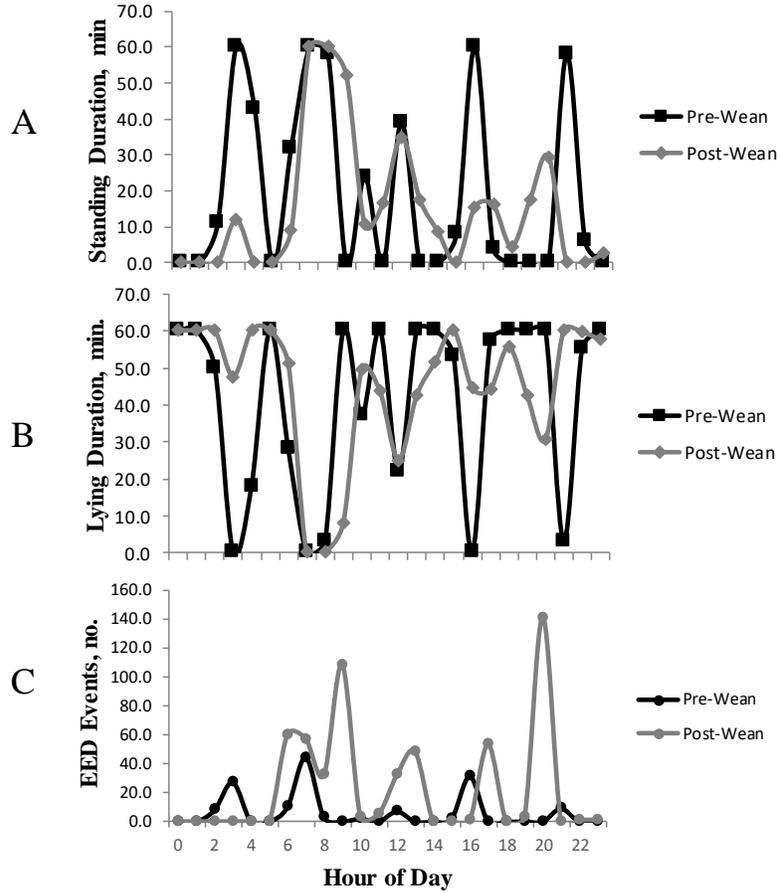


**Figure 2-11.** A. “Non-nutritive oral behaviors (NNOB)” are timestamped when the calf is licking, biting, or sniffing an object, grooming, and head-butting or sucking objects other than the environmental enrichment device (EED) or milk bottle. B. Milk drinking behavior is timestamped when milk bottle is present during morning and evening feedings. C. “Environmental enrichment device (EED)” is timestamped when the calf contacts the dummy nipple. This behavior is not timestamped when a milk bottle is in place of the EED during morning or evening feeding times. D. Feeding behavior is when the calf’s head is over or in the feed bucket.



**Figure 2-12.** A. The graph represents a calf's duration of standing (minutes) in a 24-hour period. The pre-pairing line represents duration of standing before being pair housed and post-pairing line represents standing duration after the calves are pair housed. B. The figure represents the lying duration of a calf for a 24-hour period. The pre-pairing line represents duration of lying before being pair housed and post-pairing line represents duration of lying after pair housing. C. The figure represents a calf's use of the environmental enrichment device (EED) for a 24-hour period. Pre-pairing represents use of the EED before calves are pair housed. Post-pairing represents EED use after calves are paired.

## Outdoor Housed Calf



**Figure 2-13.** A. The figure represents standing duration of an outdoor housed calf for a 24-hour period. The pre-wean line represents standing duration before weaning and the post-wean line is after weaning. B. The figure represents lying duration of an outdoor housed calf in a 24-hour period. The pre-wean line represents lying duration before weaning and the post-wean line is after weaning. C. The figure represents an outdoor housed calf's use of the environmental enrichment device (EED) in a 24-hour period. The pre-wean line represents EED use before weaning and the post-wean line is after weaning.

# **Chapter 3 - Calf Maternal Passive Transfer Immunity Measures Associated with First-Meal Suckling Behaviors and First-Week-of- Age Automated-Measures.**

## **Abstract**

In ruminant species, including cattle, initial colostrum ingestion is critical for the health of the calf. Newborn calves must first stand and be motivated to seek and successfully suckle the dam's udder. The objective of the study was to determine if calf behavior data collection techniques (both direct observation and automated) would correlate with measures of passive transfer of immunity (hematocrit; total plasma protein; Immunoglobulins, IgG1 and IgM). This study took place in March of 2018 near Pollock, South Dakota. Angus x Angus cross heifer-calf pairs (n=59) were observed for 12 hours after birth. The variables collected included: each calf's first-meal suckling behaviors; automated activity behaviors, and measures of passive transfer of maternal antibodies (IgG1, IgM, hematocrit, and total plasma protein). All correlations are Pearson correlations using SAS 9.4. There was a tendency ( $P=0.08$ ) for IgG1 concentrations to have an inverse relationship with calf body weight ( $r=-0.23$ ). Total plasma protein was correlated ( $P<0.01$ ) with IgG1 ( $r= 0.52$ ) and IgM ( $r= 0.52$ ). Likewise, hematocrit was correlated with IgG1 ( $P=0.01$ ,  $r= -0.37$ ) and IgM ( $P=0.01$ ,  $r= -0.34$ ). Total Plasma Protein had an inverse relationship with birth-to-stand intervals ( $r= -0.45$ ;  $P<0.01$ ). In addition, total plasma protein tended ( $P=0.079$ ) to decrease as the birth-to-suck interval increased ( $r= -0.24$ ). Total plasma protein increased with the number of lying-bouts per day for the first week of life ( $r= 0.23$ ;  $P<0.01$ ). Furthermore, the birth-to-last teat suckled interval tended to decrease as temperature on the day of birth increased ( $r= -0.24$ ;  $P=0.08$ ). Lying duration during the first week of life increased as the

temperature on the day of birth increased ( $r= 0.29$ ;  $P=0.03$ ). The birth-to-stand measure can be a reliable indicator of a calf's total plasma protein and give indication to transfer of immunity. The use of automated data collection can be beneficial in understanding early life behaviors in calves and aid in management decisions. Additional research is needed to better understand calf behavior in a more extensive production setting.

Key words: suckling, immunity, calves

## **Introduction**

In bovine and other ruminant species, newborns should receive a large meal of colostrum by 24 h after birth (Godden, 2008). This first meal helps the calf gain passive-transfer of antibodies from its mother. Most handfed dairy calves have blood routinely checked, and it is estimated 1 out of 4 calves have failure-of-passive-transfer (Hulbert and Moisé, 2016). In addition, dairy calf behavior data are used to determine or predict illness (Borderas et al., 2008; Quimby et al., 2001, Hulbert and Moisé, 2016). In comparison, beef cattle systems are more extensive than dairy, and the first-meal quality is more dependent on the mother-infant bond rather than the human caretaker. Also, invasive data collection techniques (i.e. blood sampling) are not always practical among beef operations. Ingestion of colostrum supplies the calf its first antibodies, primarily IgG1. Behaviors such as standing, exploring, and suckling are indicators that a calf will be more successful in obtaining their first meal (Murray and Leslie, 2013). Therefore, our objective was to determine if calf behavior data collection techniques (both direct observation and automated) would correlate with measures of passive transfer (hematocrit, total plasma protein, Immunoglobulins G1 and M).

## **Materials and Methods**

This observational study took place at a ranch near Pollock, South Dakota in March 2018. Angus x Angus cross heifer-calf pairs (n=59) were monitored continuously 24 h a day for signs of labor and observed for 12 h after parturition. Data were collected on each calf's early life behaviors including time of stand, time of first suckle, time of last teat suckled, and automated activity data. Cattle were housed in an 11-acre birthing pasture and provided water ad libitum and supplemental feed once per day. Heifers were continuously monitored by three trained observers for signs of labor progression. If there was precipitation (e.g. rain, snow), heifers (-6 to +24 hours relative to calving) were moved into a barn (non-climate controlled). The calving barn contained twelve 3.05 × 3.05 m maternity pens that were bedded with 15.24 cm of hay and cleaned daily. Calves (indoors or outdoors) were observed for 12 h after birth and direct observational data was collected. Timestamping for observational data measures included birth, first-stand, first-suckle, and last-teat suckled. Twenty-four hours after birth, a total of 4 mL of whole blood was collected from each calf. Next, calves were weighed, tagged, and fitted with a 3-axis accelerometer (Hobo Pendent G, Onset Computer Corp., Bourne, MA) on their left hind leg. A commercially available activity monitor (Fitbit Zip, Fitbit, San Francisco, CA) was fastened on their ear-tag. The leg and ear-tag loggers continuously collected activity every minute for the first 6 days of life.

Whole blood was processed using standard centrifugation and pipetting techniques. Plasma was stored at -20°C until analysis. Passive transfer measures included plasma IgG1 and IgM, which were collected using standard ELISA methods (Bethyl Laboratories, Montgomery, TX) and total plasma protein determined using a BRIX refractometer (Extech, Nashua, NH). The coefficients of inter- and intra-variation for the immunoglobulin measures were <15% and <10%, respectively. Automated behavior data were summarized by 24 h periods using a

customized spreadsheet application (DynaSim, Manhattan, KS). Descriptive statistics were generated, and Pearson correlations were determined using SAS (version 9.4., 100 SAS Campus Drive, Cary, NC).

## **Results and Discussion**

Beef calf raising systems are managed in a more extensive manner when compared to dairy calf production. The supervision of calves and their dams in beef production happens less often than dairy calves and dams. Dairy cows generally calve in a maternity pen with human supervision while beef cows calve in larger land areas. Production system variation for beef calves results in difficulties studying and understanding their behaviors, especially early in life. Early-life calf behaviors are crucial for survival and development of the calf. The objective was to determine if calf behavior data collection techniques (directly observed and automated) would correlate with measures of passive transfer of immunity.

Due to heifers calving prior to running them through a chute, the body weights and body condition scores of 11 heifers were not collected. Direct observation data were not analyzed on four calves due to clerical errors. Automated data were not collected on eight calves due to the time of calving being towards the end of the study (Table 1). There was a tendency ( $P=0.08$ ) for IgG1 concentrations to have an inverse relationship with calf body weight ( $r= -0.23$ ; Table 2). The authors expected this observation because other researchers reported similar findings with body weight and immunoglobulin concentrations (Gaspers et al., 2014). As expected, (Bender and Bostedt, 2009), TPP was correlated ( $P<0.01$ ) with IgG1 ( $r= 0.52$ ) and IgM ( $r= 0.52$ ; Table 2). Likewise, hematocrit was inversely correlated with IgG1 ( $r= -0.37$ ,  $P=0.01$ ) and IgM ( $r= -0.34$ ,  $P=0.01$ ). These findings were expected because hematocrit is a measure of hydration and influences the dilution of solid-particles in plasma (Baker, 1989). Total Plasma Protein had an

inverse relationship with birth-to-stand intervals ( $r = -0.45$ ;  $P < 0.01$ ; Table 2). The longer the calf took to stand after birth, the lower the total plasma protein. In addition, TPP tended ( $P = 0.079$ ) to decrease as the birth-to-suck interval increased ( $r = -0.24$ ; Table 2). Tight-junctions in the gut that allow passive transfer start closing within 4 hours after birth (Stott et al., 1979). The birth-to-stand interval is likely dependent on the amount of time it takes the calf to gain access to colostrum. Some dairy cattle researchers use a scoring system for calf vitality (Murray and Leslie, 2013; Mee, 2008b), and standing is part of the scoring process. However, scoring methods require trained observers and greater error between people is expected compared to time-based measures (Martin and Bateson, 2007), and therefore, time-based measures (i.e. birth-to-stand measure) are more likely to serve as an accurate biomarker for passive transfer. Furthermore, a Chi-square analysis of teat preference on first suckle by the calf showed 40% of the calves used the front-left quarter first followed by 27.3% front-right, 20% back right, and 12.7% back-left (Figure 1). Selection of a front teat could be a result of access to the udder being easier in the front half of the udder due to dam's hind legs blocking the back half of the udder more. A calf seeking the udder may locate a hind limb of the cow and follow it up till coming in contact with the udder which potentially presents the front half of the udder first. In addition, front teats of the udder may have a greater distance from the ground compared to the back teats making it easier for the calf to find and suckle (Ventorp and Michanek, 1992). The dam's natural flight zone may also result in the calf's location being near the dam's side rather than more posterior due to the presence of a blind spot directly behind the cow (Grandin, 2014).

Environment also plays an important role in these behaviors. Environmental stress including cold and wet conditions can influence colostrum ingestion and subsequent blood components, including immunoglobulin concentrations and total plasma protein.

Immunoglobulin (G1 and M) concentrations (Table 1) of calves reflected similar values for two-year old heifers from previous studies (Odde, 1988). Cold stress was associated with variation in colostrum intake and subsequently, increased FTP-rates (Olson et al., 1980). In addition, the environment influences the progression of labor and ultimately, calf vitality (Barrier et al., 2012). The birth-to-last teat suckled interval tended ( $P=0.08$ ) to decrease as temperature increased on the day of birth ( $r= -0.24$ ; Table 2). Dairy calves have been shown to be more active during summer conditions compared with winter conditions, especially lying time (Tripon et al., 2014). The observed result shows calves are more active during warmer temperatures on their day of birth leading to a tendency for calves to nurse all quarters of the udder with a reduced duration. Calves born on days with lower temperatures may experience cold stress leading to reduced precocious behaviors after birth, which may hinder their passive transfer of immunity. Healthy calves are generally more active, which increases heat production and helps with cold weather conditions (Vermorel et al., 1989b). Thermogenesis in calves experiencing dystocia can be up to 36% lower than calves that did not experience dystocia (Vermorel et al., 1989a). Furthermore, no other measures including heifer measures, calf measures of health, birth-to-stand and suckle, udder use, lying bouts, and head movement had relationships with temperature and/or wind speed on the day of birth.

Interestingly, TPP increased with the number of lying-bouts per day for the first week of life ( $r= 0.23$ ;  $P<0.01$ ). This may indicate that other immunoglobulins and factors from colostrum help the calf be more active (Quigley and Drewry, 1998). Another explanation may be that calves are more precocious and innately skilled at suckling will likely be more motivated throughout the day to interact with the dam (Ventorp and Michanek, 1991; Godden, 2008). Furthermore, lying duration during the first 6 days of life increased as the temperature increased

on the day calves were born ( $r= 0.29$ ;  $P=0.03$ ; Table 2). Figure 2 represents data collected from automated devices (3-axis accelerometer and activity monitor) for calves' behavior's during the first week of life. The figure includes graphs of lying duration (mean=1,016.6 min or 16.9 h; SD= 67.3 min or 1.1 h; Table 1), lying bouts (mean= 28.7; SD= 7.0; Table 1), and head movement (mean= 14,729.6; SD= 4,801.9; Table 1) of calves fitted with automated devices. These automated measures provide more information about calf activity because minimal labor is needed. Future studies will incorporate a directional accelerometer that fastens to the ear-tag and can collect both stand-rest postures and head-activity. The drawback is that devices need placement after the first-meal is completed to not disrupt the mother-infant dyad. Consequently, activity in the first hours of life need direct observation. Therefore, the authors recommend both direct measures and automated data for the first week of life.

### **Conclusions and Implications**

Neonatal intake of colostrum influences the animal's lifetime productivity (Faber et al., 2005). Beef cattle producers may want to focus on producing a calf with greater vitality or vigor than dairy cattle producers because of their more extensive systems. A precocious calf will stand up and nurse its mother sooner than a less precocious calf. Calves need to nurse their mother soon after birth to receive adequate colostrum and passive transfer of immunity (Godden, 2008). Total protein plasma is a very low-cost blood measure and correlates with Ig concentration. Occasional or routine refractometer plasma measures may help producers determine the quality of suckling for the first-meal. Behavior measures can serve as less invasive techniques to determine passive transfer of immunity status because of nursing-quality measure and first-of life measure. Direct observation requires a skilled, and patient caretaker to collect the birth to suckle measure, however, the birth-to-stand measure is more correlated with TPP. The authors found

that birth-to-stand measure is a time-efficient direct observation associated with TPP. This work also indicates that automated data may help producers make choices about the vitality or vigor of their calves in the first week of life.

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**Table 3-1. Descriptive statistics of heifers and calves and replicates needed for future studies.**

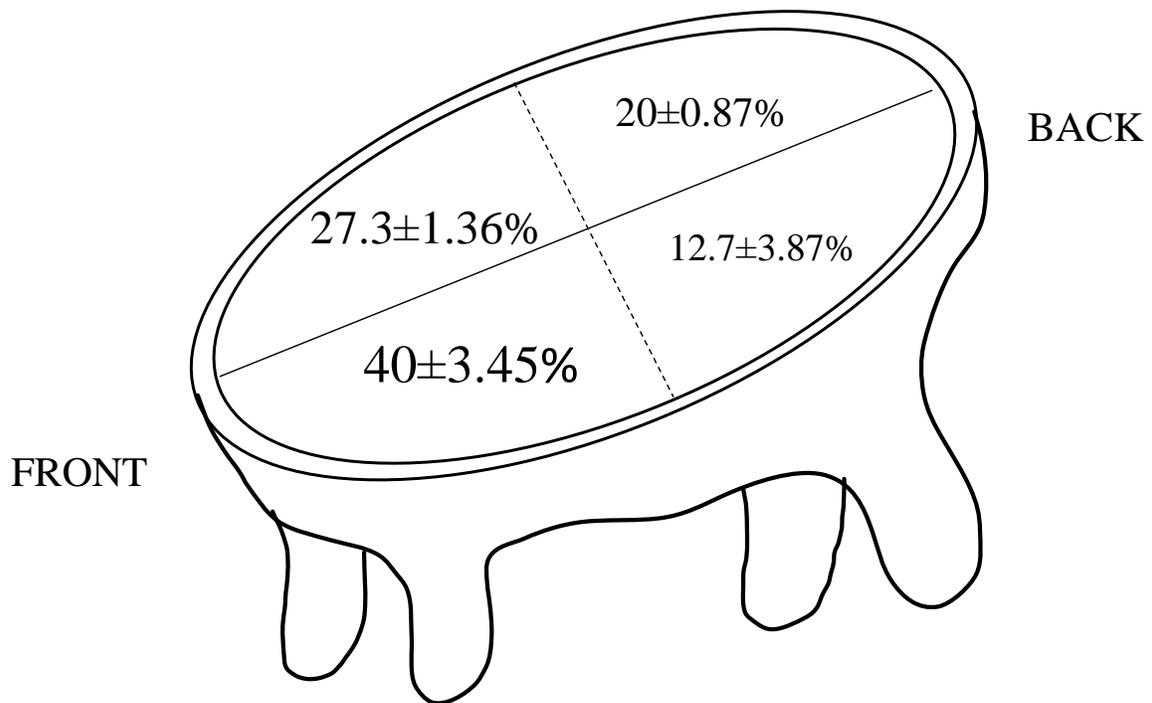
	No. Obs.	Descriptive statistics for heifers and calves								No. experimental units to detect % from $\alpha = 0.05$ $\beta = 0.10$	
		Mean	Median	Min	Quantiles, %			Max	CV%	50%	
					25	50	75				
<i>Heifers Information</i>											
Gestation length, days	59	276.3	276.0	270.0	273.0	276.0	279.0	288.0	1.3	--	
Heifer pre-calving weight, kg	48	583.2	580.6	499.0	561.3	580.6	602.1	696.3	6.3	--	
Heifer body condition score	48	6.4	6.0	5.0	6.0	6.0	7.0	7.0	8.9	--	
<i>Calf measures of health</i>											
Calf birth weight, kg	59	33.8	33.6	24.5	30.4	33.6	36.3	43.1	13.5	2	
Total Plasma Protein, %	59	9.5	10.0	5.0	8.0	10.0	11.0	14.0	20.2	3	
IgG, mg/mL	59	25.2	13.8	1.5	9.2	13.8	31.8	95.0	96.4	78	
IgM, mg/mL	59	1.6	1.0	0.03	0.4	1.0	2.0	9.3	117.6	116	
Hematocrit (HCT),%	59	37.7	35.9	22.4	33.8	35.9	41.5	53.8	16.7	2	
<i>Calf Behaviors at first-nursing</i>											
Intervals (minutes) of Birth-to-											
Stand	55	63.1	40.5	10.0	23.9	40.5	87.0	246.7	88.7	66	
First suckle	55	117.6	81.5	23.6	48.2	81.5	145.4	502.0	83.5	59	
Last teat suckled	55	149.8	114.4	38.8	70.7	114.4	183.8	652.9	81.5	56	
Udder use, %	55	90.0	100.0	25.0	100.0	100.0	100.0	100.0	21.7	4	
<i>Calf activity after nursing</i>											
Lying bouts number/day	51	28.7	28.9	13.6	23.8	28.9	33.3	46.0	24.4	5	
Lying duration, minutes/day	51	1016.6	1013.2	885.6	971.9	1013.2	1055.9	1224.8	6.6	--	
Head movement, number/day	32	14729.6	14876.3	5988.0	10974.4	14876.3	17933.0	25833.7	32.6	9	

**Table 3-2. Pearson’s correlations of calf behaviors, passive transfer of immunity measures, and environment conditions.**

	n =	TPP		IgG1		IgM		HCT, %		Temperature <sup>1</sup>		Wind Speed <sup>2</sup>	
		r	P =	r	P =	r	P =	r	P =	r	P =	r	P =
<i>Heifers Information</i>													
Gestation length, day	59	0.12	0.38	0.07	0.59	- 0.02	0.85	-0.18	0.17	--	--	--	--
Heifer pre-calving weight, kg	48	0.01	0.98	0.05	0.75	0.11	0.44	-0.18	0.22	--	--	--	--
Heifer body condition score	48	-0.14	0.34	0.22	0.13	0.12	0.41	-0.19	0.20	--	--	--	--
<i>Calfmeasures of health</i>													
Calf birth weight, kg	59	0.01	0.97	-0.23	0.08	-0.2	0.14	0.16	0.22	-0.06	0.67	0.11	0.41
Total Plasma Protein (TPP), %	59	--	--	0.52	<0.01	0.52	<0.01	0.07	0.62	-0.01	0.94	0.02	0.86
IgG1, mg/mL	59	0.52	<0.01	--	--	0.89	<0.01	-0.37	0.01	0.16	0.24	0.13	0.34
IgM, mg/mL	59	0.52	<0.01	0.89	<0.01	--	--	-0.34	0.01	0.08	0.53	0.10	0.45
Hematocrit (HCT), %	59	0.07	0.62	-0.37	0.01	-0.34	0.01	--	--	-0.05	0.73	-0.15	0.25
<i>Calfbehaviors at first-nursing</i>													
Intervals (minutes) of Birth-to-													
Stand	55	-0.45	<0.01	-0.16	0.24	-0.09	0.52	-0.2	0.16	-0.01	0.95	0.11	0.42
First suckle	55	-0.24	0.08	-0.21	0.12	-0.2	0.15	-0.16	0.26	-0.02	0.90	-0.14	0.32
Last teat suckled	55	-0.17	0.22	-0.10	0.47	-0.11	0.44	-0.23	0.09	-0.24	0.08	-0.10	0.47
Udder use, %	55	0.08	0.55	0.14	0.30	0.17	0.22	-0.07	0.62	--	--	--	--
<i>Calfactivity after nursing</i>													
Lying bouts, number/day	51	0.23	<0.01	0.05	0.34	0.08	0.15	-0.02	0.75	-0.07	0.63	0.11	0.45
Lying duration, minutes/day	51	-0.01	0.92	0.01	0.94	0.01	1.00	-0.06	0.28	0.29	0.03	0.06	0.67
Head movement, number/day	32	-0.06	0.74	-0.16	0.38	-0.11	0.56	0.07	0.70	-0.03	0.88	0.19	0.30

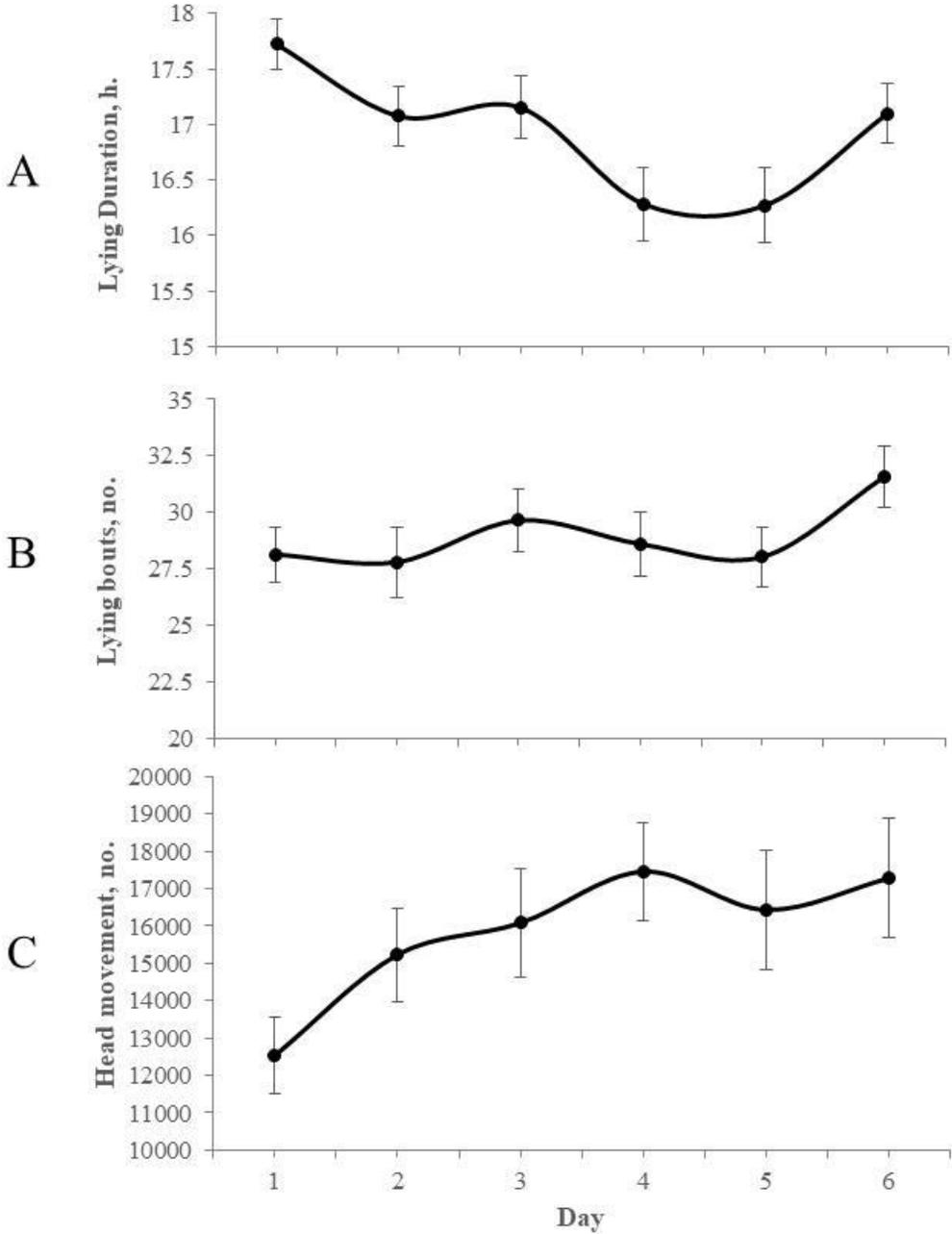
<sup>1</sup>Average outside temperature on the day the calves were born

<sup>2</sup>Average wind speed on the day the calves were born



**Figure 3-1.** Chi-square analysis of first teat suckled by calves. Values represent the percent of calves using 1 of 4 teats (front-left, front-right, back-left, and back-right). Chi-square statistic of 36.87 and  $P < 0.01$ . Expected totals for front-left, front-right, back-left, and back-right were 26.8, 22.4, 27.7, and 23.2%.

**Figure 3-2.** A. Graph represents the lying duration (h) for calves during the first 6 days of life. B. Graph represents the number of lying bouts (no.) the calves had during the first 6 days of life. C. Graph represents the head movement of the calf (no.) during the first 6 days of life.



## **Chapter 4 - Conclusions/Implications**

Applied ethologists express great concern for understanding animal needs that are under human management. The use of observational data is important for developing methods to better quantify behavior of animals in confinement. Scientists can make more definitive conclusions about animal behavior through the use of automated data collection methods. The understanding of animal needs and welfare benefits from expansion of knowledge in regards to animal behavior. Applied ethologists focus on improving animal welfare and accordingly, decrease the public's perception of livestock production being "bad." Concern for animal production has led to increases in legislation being directed to changing animal living conditions under confinement (Matheny and Leahy, 2007). Legislation regarding animal welfare has increased the need for applied ethologist to alter and advance technologies for collecting data without increasing distress experienced by the animal. In calf production, especially dairy, welfare of the calf is an increasing topic today due to the decrease in "natural" living of the calf (Stull and Reynolds, 2008; Keyserlingk et al., 2009; Vasseur et al., 2010). The living conditions calves are raised in could benefit by previous work involving environmental enrichment, space allowance, and socialization, while giving applied ethologist data to quantify and make changes to areas of production with animal welfare problems. Technologies including video recording systems, activity monitors, and environmental enrichment devices are toolsets that can be used in various animal production systems.

Applied ethologists, farmers, and animal caretakers can use automated toolsets (video recording systems, activity monitors and environmental enrichment devices) to determine normal/abnormal behaviors and patterns of calves without increasing unnecessary handling.

These technologies can be used continuously without the need of a technician or worker to maintain them constantly. Video recording systems are being used more often than ever in the dairy industry as well as other facets of animal production (e.g. slaughter facilities). The benefit of these systems is they provide increased control over an entire production system. Producers can rely on the systems to calculate where production is being held up and determine if unnecessary handling techniques are taking place. In addition, using video recording systems encourages animal caretakers to be held at a higher standard and could result in a reduction of incidences on farm regarding animal welfare. Increased occurrences of inhumane handling and living conditions for livestock have introduced avenues for supervision of livestock production by consumers, producers, and scientists.

The restriction of natural suckling presented in dairy calf production may be remedied through novel application of environmental enrichment devices. Implementing such technology may also aid in the reduction of negative publicity on management practices for dairy production. Separation of dairy calves at birth may appear cruel but is necessary to prevent transmission of disease and reduce mortality in newborn calves (Davis et al., 1954; Lorenz et al., 2011). Developing technologies such as an environmental enrichment device allow a calf to perform natural sucking behavior while providing scientists and producers with quantifiable data. Data provided by automated technologies (e.g. EED and rest logger) could contribute to earlier detection of abnormal behaviors and illness allowing for a more effective treatment time (Weary et al., 2009). Automated technologies are less-time consuming compared to live or video-based observations and can give beneficial measures of health and welfare (Rushen et al., 2008). Furthermore, training individuals to monitor and categorize animal behavior can be costly and time consuming in research and/or production settings. The increase in use of automated

measures allows for more accurate data collection and could reduce labor and time needed to collect data.

Animal scientist and producers understand the importance of defining normal from abnormal behaviors in calves. The restriction of suckling behaviors in dairy calves may lead to the development of abnormal oral behaviors. Abnormal oral behaviors (e.g. cross-sucking, self-sucking, milk stealing, and tongue-rolling) may be a result of the lack of expression of natural sucking by the calf. The motivation to suck occurs frequently after a milk meal in dairy calves and is stimulated by ingestion of milk (Passillé, 2001). Incorporation of an environmental enrichment device, especially after a meal, allows the calf to perform sucking behaviors necessary for development and satiation of the behavior. The device could give crucial information on calf performance, health, and well-being when baseline measures are used to monitor animals. Elimination or reduction of abnormal behaviors is critical in dairy calves before being moved to group living. Calves that express abnormal oral behaviors in individual housing are likely to express those same behaviors in group housing and adulthood (Latham and Mason, 2008). Abnormal oral behaviors like milk stealing or self-sucking could lead to decreased production and in extreme cases culling of the animal. Dairy cows are a large investment for dairy farmers and culling of animals is of last resort but necessary for maintaining specified production values. Using environmental enrichment could lead to a decrease in unwanted behaviors early in life allowing for a better transition into the cow herd later in life.

Furthermore, activity monitors have been established in research and production with respect to determining daily activity patterns of livestock. Activity monitors in dairy cattle production provide data on the animal's active and inactive behaviors such as locomotion, resting, and rumination. Automated activity data gives valuable information about the welfare

and health of dairy cattle. Lying patterns in dairy cattle can be used to detect illness, estrus, and locomotion problems (Rutten et al., 2013). Utilization of activity monitors in research allows scientist the ability to collect more measures for analysis without an increase in invasiveness experienced by the animal due to easy application of monitors. The application of automated devices can be used in a variety of settings including more extensive production systems such as beef cattle. Implementing these devices in a range beef cattle setting allows for better understanding of their behaviors in a grazing environment. Live observation of animals in a grazing setting can be almost impossible at times due to the large land area cattle are raised in but automated measures could be more practical in these situations.

In conclusion, applied ethologist and producers are continuously looking for ways to improve animal health, performance, and welfare. Rising demand from consumers in regards to animal welfare results in decision making by scientist and producers to find better methods for understanding what animals need. The study of animal behavior gains insight using automated technologies (e.g. environmental enrichment device, activity monitors, video recording systems, etc.) without increasing distress experienced by the animal. Furthermore, implementation of such technologies reduces labor and time associated with monitoring of animals through live observation by reducing the need for live observation. Data collected from newer technologies aids in categorization of animal behavior with more specified parameters by increasing accuracy and sensitivity. Human error in behavioral data collected with automated techniques can be significantly decreased compared to live observation techniques. Future studies and animal husbandry will continue to introduce automation as technology improves, becomes more user friendly, and affordable and ultimately leading to improvement in management of livestock production.

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