Applied Ethology Management Methods for Resilient Calves

by

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Abstract

Behaviors and immune-measures can be used as indicators of animal health and welfare. Measures of passive immune transfer and solid-feed intake often are used to gauge weaning readiness for dairy cattle. Technological improvements in an automated collection of behavioral data make it feasible to replace invasive techniques and time-consuming measures. This thesis will introduce studies that used applied ethology for beef and dairy calves as indicators of resilience, passive immune transfer, and weaning readiness.

Study 1 was conducted to determine if automated data collection of stand-lie behaviors and environmental enrichment device (EED) usage could detect differences among four different weaning protocols for male Holstein calves. For first treatment, MOD-STEP calves were fed 0.66 kg/d of milk replacer (MR) and were step-down weaned by age 6 weeks (PM milk replacer feeding was withdrawn 1 wk before weaning, and last milk replacer feeding was withdrawn at age 42 d). For the remaining 3 treatments, calves were fed higher planes of milk replacer (1.09 kg/d MR). Treatments were: 1) Step-down weaned (HI-STEP) at age 6 wk; 2) Step-down weaned at age 8 wk (HI-LATE), or; 3) Gradually weaned by age 8 wk (HI-GRAD). From age 6 d to 1 wk after weaning, calves were provided an environmental enrichment device (EED), which was a dummy nipple attached to a bottle and holder. In addition, calves had an accelerometer attached to their rear leg to detect stand-lie data before, during, and after weaning. Results showed that calves fed HI-milk replacer (HI-STEP and HI-GRAD) used EED with more frequency and spent less time resting. This study confirms that applied ethology can be used as an indicator of "weaning readiness" in dairy calves.

Study 2 was conducted to identify and refine directly observed calf nursing behaviors and better understand their relationships to physiological biomarkers in dams and calves. Data from

59 two-year-old Angus-cross heifers' body weight and blood were collected during day one of the study. Heifers were then moved to a maternity pasture where trained observers monitored calving progression. Times were collected for each calf's: birth (calf on the ground); stand (all four limbs upright for > 5 seconds); first-suckle (mouth contact with any teat); and each teat during 24 hr after birth. After the 24 h observation period, body weights were measured, and blood was collected and used to measure complete blood counts. Plasma was analyzed for: immunoglobulins G1 and M, total plasma protein (TPP), cortisol, and haptoglobin. Calves were divided in two groups using a threshold of 10 g/L of IgG. Calves that failed to acquire passive immune transfer required more time to stand up and to start suckling. Correlations were found between TPP and latency to stand and to first suckle, indicating that precocious behaviors can be used to predict passive immune transfer in neonatal calves.

Applied ethology tools can be used to refine and replace invasive and time-consuming measures, such as blood collection and solid feed weigh back. Ethology also may be used guide calf raisers' management decisions.

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Chapter 1 - Review of Literature

Introduction

A neonatal calf is referred to period of birth until its first 28-30 days of life. During this period, proper care and good welfare are extremely important for calf health. Intense care can prevent early morbidity and high mortality. Leading factors associated with neonatal mortality are failure of passive transfer (FPT; Weaver et al., 2000) and dystocia (Odde, 1996; Laster and Gregory, 1973). Although failure of passive transfer is not a disease, it makes calves susceptible to develop infectious disorders (Weaver et al., 2000) such as diarrhea, respiratory diseases, and omphalophlebitis (Mee, 2013; Besser and Gay, 1994). During the first hour of life, mortality risk was estimated to be approximately 69% (Bellows et al., 1987) for beef calves and 75% for dairy calves (Mee, 2013). These calf deaths cause a significant economic loss to producers. The USDA in 2011 estimated 13.1% of the non-predator loss in U.S. is due to calving problems. Management during parturition can help prevent exposure to pathogens, decreasing risk of infections (Smith, 2007). Dystocia and failure of passive transfer are the most common factors associated to neonatal calf losses (Mee, 2004; Odde, 1996). These two factors can be decreased by improving management of the farm. Although there is more attention paid to management of neonates' environment, cow and calf behavior are emerging as a toolset to predict health and immune resilience in calves (Hulbert et al., 2019; Hulbert and Moisa, 2016). Knowledge of natural behaviors is important to identify abnormal behaviors throughout different species. Maternal behaviors such as licking a calf will promote cardiovascular and respiratory stimulation of a neonatal calf (Von Keyserlingk and Weary, 2007; Edwards and Broom, 1982). A vigorous calf will stand up and start suckling colostrum with a few hours after birth (Odde et al., 1985).

This innate behavior is extremely important for calf to acquire enough colostrum to ensure a successful passive transfer, especially when they are left to suckle their dams (Besser et al. 1991). Behavior studies are necessary to improve managements practices related to neonatal calves, but it is time consuming when data is acquired from live observations. More recently, video cameras and automated devices such as loggers are being used to acquire remote behavioral data with more precision and disturbing the least researched animals (Bonk et al., 2013; Ledgerwood et al., 2010).

Pre-Parturition

Cow overall health

The overall health during pregnancy and parturition need to be accounted for calf health and survival. Homeostasis and homeorhesis are involved in control of cow body metabolism during pregnancy and lactation. During gestation, fetus growth and mammary gland development will be prioritized, and considerable a part of maternal nutrients will be used for their maintenance (Picciano, 2003; Bauman and Currie, 1980). Energy requirements for a pregnant cow will be 75% greater than for a non-pregnant cow (Bauman and Currie, 1980). During this time, it is essential that cows are fed properly to maintain a desired body score. For first-calf heifers, a body condition between 5.5 to 6.0 at calving is recommended (Odde, 1996). This recommendation is given once what cows and heifers are fed prior to parturition influences calf survival. Researchers reported that calves born to heifers with lower body condition (< 5; scale of 1 = emaciated to 9 = obese; Richards et al., 1986) have a lower vitality and consequently a lower passive transfer (Odde, 1996; Carstens et al., 1987).

Parturition

Environmental conditions

Environmental conditions where calves are born are variable, and dependent on weather, available facilities, and farm managements. Heifers and cows are usually moved to maternity pastures prior to their calving date. This practice will decrease calves' exposure to pathogens once they are born (Smith, 2007). In cold weather, it is common to move dams to maternal pens inside barns, especially heifers because they require more assistance than cows (Odde, 1996). Although these pens allow dams to be closely monitored during their stages of parturition, pathogen exposure is also greater (Odde, 1996). Environmental conditions such as temperature and moisture can affect the ability of neonatal calves to resist disease and can influence pathogen replication, increasing risk of infections (Smith, 2007). A well-managed environment will decrease exposure to infectious pathogens and prevent future calf loss. Weather conditions at calving time may also affect calf vitality and behavior (Smith, 2007). During early neonatal periods, calves are predisposed to thermolysis to adapt to extra-uterine life (Carstens, 1994). Calves born in warm weathers tend to be more active, standing up quickly and suckling colostrum easily, while calves born in harsh cold and high humid conditions have difficult time keeping body temperature homeostasis. Carstens (1994) reported that calf mortality progressively increases as environmental temperature decreases or precipitation increases. Calves are born covered in placental fluid which can freeze within seconds after they are born in negative temperatures. In addition, calves exposed to severe cold during prolonged hours can deplete their energy reserves, inducing physical weakness, and can have a decrease in immunoglobulins absorption, leading to mortality (Carstens, 1994). Cold weather can also make

calves more susceptible to “weak calf syndrome”, a condition where calves show muscle weakness and lethargy. They also take longer to stand up and suckle (Stauber, 1976).

Dystocia

In order to deliver a health and viable calf, management of cows is undoubtedly important. Dystocia and perinatal mortality are the leading problems at calving time (Mee, 2004; Odde, 1996) and comprise 16% of periparturient disorders of U.S. dairy herds (Mee, 2004). In addition, dystocia increases neonatal calf disease susceptibility and mortality (Andersen et al., 1993). First-calf heifers are more susceptible to dystocia than pluriparae cows, especially due to fetal-pelvic incompatibility (Mee, 2004; Andersen et al., 1993). Dystocia is considered a painful condition (Huxley and Whay, 2006) and its causes can differ between groups. In first-calf heifers, the most relevant causes in crescent order are incomplete dilation of the cervix and vulva, abnormal fetal disposition, and feto-pelvic incompatibility (Mee, 2004; Andersen et al., 1993). Researchers attribute the most important factor in dystocia as the proportion between a dam’s pelvic area and fetus birth weight (Andersen et al., 1993). While in cows the most common causes are incomplete dilation of the cervix and vulva followed by uterine torsion, uterine inertia, twinning, fetal-pelvic incompatibility, and abnormal fetal disposition. Dystocia is also considered a risk factor for neonate respiratory acidosis, which later can affect acquisition of passive immunity (Quigley and Drewry, 1998).

Calf immunologic development

Fetal and neonatal calf immune defenses

Calves immunology starts to develop *in utero*, immune and non-specific mechanisms are built to promote calves’ defenses. Stomach acids, enzymes secretions, and microbiota in mucosal

tissues are anatomic barriers and examples of non-specific defenses in neonatal calves (Barrington and Parish, 2001). Neutrophils and macrophages are also non-specific defenses that contribute to early fetal protection, although they are only released into the blood later in gestation (Banks, 1982). When calves are born, nonspecific defenses are functional; however, they can be suppressed by stress, malnourishment, mild infections, and toxins exposures (Barrington and Parish, 2001). Immune defenses are developed and increased during the course of gestation, including lymphocytes (T and B), antibodies, and effector cells. Lymphocytes will migrate from their original mature sites: thymus, bone marrow, and Peyer's patches to lymphoid organs during the first trimester of gestation (Barrington and Parish, 2001). Although calves have lymphocytes, they still lack immunocompetence (Hulbert and Moisá, 2016), which is the reason why Schultz et al. (1972) suggested that antigenic stimulation is required to induce morphological and functional immunological activity in the fetus. To prove this hypothesis, researchers studied *E. coli* in utero vaccination that showed increased production of fetus antibodies (Conner et al., 1973; Gay, 1971). Calves born to prenatal vaccinated cows had IgG, IgM, and anti-*E. coli* defenses in duodenum, jejunum, ileum, and jejunal lymph nodes (Conner et al., 1977). Although nonspecific and immune defenses are present in newborn calves, they are still exposed to new environment and unfamiliar microorganisms at calving time. Neonatal calves are considered to be agammaglobulinemic (McGuirck and Collins, 2004) and their immunity relies mostly on complement components (Banks, 1982) that will enhance activity from phagocytic cells such as neutrophils. A newborn calf will have larger number of neutrophils than an adult cow during its first days of life (Rossi et al, 1979). During the immune maturation phase, passive immune transfer provided from colostrum promotes protection to calves (Gomes et al., 2014).

Passive immune transfer

The importance of passive immune transfer is very well discussed, especially in ruminant species due to their synephiteliochorial placentation that is impermeable to macromolecules passage. Therefore, colostrum is the first mammary secretion after birth and supplies immunoglobulins, immune cells, growth factors, lactoferrin, nucleosides, vitamins, peptides, lysozyme, oligosaccharides, cytokines, and nutrients to neonatal calves (Gapper et al., 2007; Barrington and Parish, 2001). Colostrogenesis starts a few weeks prior and terminates shortly before parturition (Barrigton et al., 1997; Sasaki et al., 1976; Pierce and Feinstein, 1965; Larson, 1958). Immunoglobulins are derived from blood or produced by intra-mammary plasma cells (Stelwagen et al., 2009) that will later be transported to colostrum by an active receptor in alveoli cells (Besser and Gay, 1994). These immunoglobulins are the main immune component present in colostrum (Stelwagen et al., 2009) and their absorption is crucial to provide passive immunity to neonatal calves (Pakkanen and Aalto, 1997). The most predominant immunoglobulin is IgG1, comprising approximately 80% of total bovine colostrum components (Larson, 1958; Pierce and Feinstein, 1965; Sasaki et al., 1976; Barrigton et al., 1997), whereas IgG_2, IgA, and IgM are also present but in lower concentrations (Besser and Gay, 1994; Larson et al., 1980). Because of its higher concentration, IgG1 is frequently used as efficient proof of passive transfer (Godden, 2008; Besser and Gay, 1994).

After colostrum ingestion, a non-selective macromolecular transport system in the small intestine will absorb immunoglobulins from colostrum and forward them to blood (Stelwagen et al., 2009; Pakkanen and Aalto, 1997). This transfer occurs within 24 hours after birth (Moore et al., 2005; Pakkanen and Aalto, 1997; Besser and Gay, 1994) and its absorption decreases significantly within 12 hours after birth (Stott et al., 1979). It is important to emphasize that after

being absorbed, immunoglobulins are the main agents protecting gastrointestinal mucosa against pathogens (Gapper et al., 2007). Management of passive transfer differs between dairy and beef cattle. In the majority of dairy cattle operations, heifer calves are hand-fed colostrum (53.2%; USDA, 2014) when calf is removed from its dam within 2 hours after birth (McGuirk and Collins, 2004). From those hand-fed calves, around 87.4% are bottle-fed, 8.1% are fed using an esophageal tube, and 4.5% are bucket-fed (USDA, 2014), while beef calves are usually allowed to seek and suckle a dam's teat to ingest colostrum (Von Keyserlingk and Weary, 2007; Besser and Gay, 1994).

Failure of passive immune transfer

Many factors can be associated with failure of passive transfer (FPT). In dairy calves FPT is considered when serum IgG is lower than 10 g/L (Elsohaby et al., 2015; Deelen et al., 2014; Morril et al., 2013; McGuirk and Collins, 2004). The major critical factors are quality of colostrum, adequate volume fed, and timing of colostrum ingestion (McGuirk and Collins, 2004). Factors affecting colostrum quality can also be related to cow such as parity, breed, vaccination, and health (Stelwagen et al., 2009; McGuirck and Collins, 2004; Quigley and Drewry, 1998). Volume of colostrum ingested by the calf is very important to ensure success of passive immune transfer. Commonly, a calf needs at least 0.15-0.2 kg of IgG to achieve appropriated passive transfer (Godden, 2008). This can be reached with 3 to 4 liters of high-quality colostrum (McGuirk and Collins, 2004). Researchers have reported that calves left to suckle colostrum from a dam have fewer immunoglobulins than hand-fed calves (Besser et al. 1991; Brignole and Stott, 1980). A survey from USDA 2014 showed the majority of dairy operations (87.5%) fed at least 3.7 liters of colostrum to heifer calves during first 24 hours of life, representing an increase of almost 48% from data reported in their previous survey (USDA,

2002). That shows greater concern and a better knowledge of passive immune transfer success. High-quality colostrum should have more than 50 mg/mL of IgG, less than 100,000 cfu/mL of total bacteria, and less than 10,000 fecal coliforms (McGuirk and Collins, 2004). An alternative when these standards cannot be reach is to use a colostrum replacer (Lago et al., 2018) or colostrum from donor cows properly tested and well managed (McGuirk and Collins, 2004). The USDA (2014) recommends usage of colostrum from only one cow, or pasteurized, colostrum from a greater number of cows. According, to Besser and Gay (1994), beef breeds have higher immunoglobulin concentration than dairy breeds. However, if colostrum is unavailable or deficient, dairy cows' colostrum can be used to feed beef calves to ensure success of passive immune transfer.

Non-immune factors in colostrum

Although immunoglobulins are the most predominant component, there are also non-immunoglobulin factors present in colostrum. These factors can exert bacteriostatic and bactericidal activities and help protect neonatal calf from infectious diseases (Hooijdonk et al., 2000). Lactoferrin and lactoperoxidase are the predominant non-specific antimicrobial components in bovine milk and colostrum (Hooijdonk et al., 2000). Lactoferrin causes depletion of iron from the intestinal environment, inhibiting bacteria growth and preventing neonatal infections in calves (Salmon et al., 2009; Hooijdonk et al., 2000; Pakkanen and Aalto, 1997). Nevertheless, lactoferrin plays many different roles in cellular defense, such as regulation of macrophages activity, proliferation of lymphocytes, and potentization of polymorph nuclear neutrophils bactericidal activity (Hooijdonk et al., 2000). In addition, this molecule also alters lipopolysaccharide membrane permeability, when bind directly to microbial membrane, inducing microorganism death (Adlerova et al., 2008; Hooijdonk et al., 2000). On the other hand,

lactoperoxidase *in vitro* studies showed bacteriostatic, bactericidal, and antiviral activities (Hooijdonk et al., 2000) due to production of a toxic intermediary oxidation product that inhibits microorganisms' metabolism (Pakkanen and Aalto, 1997). Because of its bactericidal activities, lactoferrin and lactoperoxidase are commercially extracted from milk to be used in food preservation, oral care, and cosmetics products (Stelwagen et al., 2009).

Cow and calf behavior

Cow maternal behaviors

Cows' maternal behaviors are believed to be driven by hormonal changes, though research about these correlations are limited. Changes in progesterone, estrogen, testosterone, prolactin and oxytocin levels are reported to be associated with aspects of maternal behaviors (Von Keyserlingk and Weary, 2007). Hours prior to calving, cows leave their gregarious natural state to seek isolation and a nesting site (Von Keyserlingk and Weary, 2007). Increased activity, standing and lying behaviors, and vocalizations are perhaps due to discomfort around calving time (Huzzey et al., 2005; Houwing et al., 1990; Edwards and Broom, 1982). Additionally, it is common for cows to calve in recumbence and they might be reluctant to stand up to lick the calf after a difficult parturition (Edwards and Broom, 1982), especially heifers that usually require more assistance than cows (Houwing et al., 1990). Cows usually stand up faster and spend more time licking the calf. Edwards and Broom (1982) reported that cows spend around 30-50% of the first post-partum hour licking their calves, stimulating calves cardiovascular system, promoting defecation and urination (Von Keyserlingk and Weary, 2007), cleaning fetal membranes, and promoting drying of the calves' coat (Edwards and Broom, 1982). This behavior is also associated with offspring recognition using olfactory-gustatory stimuli to create

a dam-offspring bond (Grau, 1976). Additionally, cows and heifers can perform placentophagia (i.e., placental ingestion) and move their calves away from the nesting site to reduce risk of predation (Edwards and Broom, 1982; Kristal, 1980). Although the previous statement seems to be reasonable regarding placentophagia, many other hypotheses for this behavior have been discussed. Some researchers attribute placentophagia to a transition to carnivore state right after calving or simple hunger (Kristal, 1980).

Neonate calf behaviors

In time following calving, neonate calves first try to lift their heads and then stand using their front legs (Houwing et al., 1990). When standing behavior is mastered, calves will follow the cow and spend some time rubbing and sniffing the dam's body (Edwards and Broom, 1982). After locating the dam's udder, calves will start to head bud in search for a teat to suckle, and finally finding a teat to start suckling colostrum. According to Odde et al. (1985) on average a beef calf performs five suckles in 24 hours and can spend a total time of 46 min suckling. As mentioned previously, beef calves are usually left to suckle their dams, while dairy calves are separated from dams within 2 hours after birth. However, feeding calves using buckets are a concern because an increase in non-nutritive oral behaviors was reported in calves separated from dams within 2 hours after birth (Houwing et al., 1990). Group housed calves can start sucking on each other, increasing risk of contamination and infection among them (Veissier et al., 2002). More studies are needed to understand non-nutritive oral behaviors and how they affect calf health and immune status.

Automated behavior collection

Behavioral data collection requires great amount of time and labor. Conventionally, these collections are in person, scan sampling, or data logging from recorded videos. However,

recently automated loggers were implemented to access stand-lie behaviors in dairy cows (Ledgerwood et al., 2010) and dairy calves' activity (Bonk et al. 2013). According to Brown et al. (2013) accelerometers have been used to measure body posture and activity in more than 120 species, increase in accelerometer usage is due to their ability to provide fine-scale measurements of behavior and make it possible to collect data without any influence of human presence (Brown et al, 2013). Accelerometers even allowed measurement and differentiation between grazing and browsing in goats (Moreau et al., 2009), while Luo (2016) reported usage of accelerometers attached to ear tags in mini-swine to collect measures of activity. Event loggers are being validated to access and measure swine and cattle oral-nasal-facial behaviors (Hulbert et al., 2019). The Onset Pendant G data logger (Onset Computer Corporation, Bourne, MA) is commonly used to collect domestic animals' behavioral data and it was reported to accurately measure lying time, laterally lying, and number of lying bouts in both dairy cows and calves (Bonk et al., 2013; Ledgerwood et al., 2010). Usage of accelerometers to measure behavior is not restricted only to terrestrial and domestic animals. Researchers reported data collection in wild and aquatic animals as well (Brown et al., 2013). This fairly new resource allowed behavioral collections that was previously considered very difficult to achieve.

Conclusion

Behavioral collection and immune-resilience are common neonatal calf topics. Previous neonatal calf researches are now becoming obsolete in virtue of all new automated technology available. Hence, there is a need to update studies and add new information that previously was very difficult to obtain. Applied ethology and immune resilience may be used as indicators to

intervene and improve management of neonatal calves, and possibly prevent morbidity and mortality the during first days of life.

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Chapter 2 - Automated-collection of environmental enrichment device and stand-lie behaviors measure weaning readiness of calves reared with four different milk-replacer and weaning strategies

Abstract

Solid feed intake is often used to determine the "weaning readiness" in dairy production. Calves fed higher (HI) planes of milk replacer nutrition consume less solid feed prior to weaning than calves fed a moderate amount (MOD) of milk replacer. Therefore, the objective of this study was to determine if automated-data collection of stand-lie behaviors and use of an environmental enrichment device (EED) could detect differences between calves on four different nutrition-weaning methods. Male Holstein calves (n = 28; 43 ± 1.2 kg BW; 1 to 2 d of age) were housed in individual pens in a naturally ventilated barn with no added heat for 56 d. All calves were initially fed one type of milk replacer (25% CP, 17% fat, 0.66 kg of DM) via nipple-buckets twice daily (0600 h and 1530 h) and one type of textured calf starter (*ad libitum*; 20% CP and 37% starch). At enrollment (age 0 d), they were randomly assigned to one of 4 nutrition-weaning methods. For the first treatment, calves were fed 0.66 kg/d of milk replacer and were step-down weaned by age 6 wk (MOD-STEP; the PM milk replacer feeding was withdrawn 1 wk before weaning and the last milk replacer feeding was withdrawn at age 42 d). For the other 3 treatments, calves were fed a higher plane of nutrition for milk replacer (1.09 kg/d MR). These treatments were: 1) step-down-weaned (HI-STEP) at age 6 wk; 2) HI-milk replacer and then step-down weaned at age 8 wk (HI-LATE), or; 3) HI-milk replacer and then gradually weaned by age 8 wk (HI-GRAD); MR reduced to 0.45 kg/d at 40 d, 0.34 kg/d at 44 d,

one 0.34 kg/d AM MR-feeding at 48 d, one 0.23 AM MR-feeding at 51 d; and all MR withdrawn at 53 d). From age 6 d to 1 wk after their weaning, calves were provided an environmental enrichment device (EED) which was a dummy nipple attached to a bottle and holder. A sensor and automated logger tracked events when each calf manipulated the EED (sensitivity was 25 Hz, event collection was 1 Hz). In addition, accelerometers were attached to the rear leg of each calf. Weaning caused calves in MOD-STEP and HI-STEP to increase solid feed intake in two steps ($P < 0.05$). While in HI-LATE and HI-GRAD, solid-feed intake increased only after weaning completion. Total lie duration, left-sided lying, and lying bouts increased among MOD-STEP calves after weaning ($P < 0.05$). For HI-LATE, HI-STEP and HI-GRAD these behaviors did not change over time ($P > 0.10$). Usage of EED did not change during weaning among MOD-STEP, HI-LATE, and HI-GRAD calves ($P > 0.10$), but EED usage increased among HI-STEP calves during weaning ($P < 0.05$). Sixteen percent of HI-STEP calves were considered frequent EED users, which was greater than expected. Behaviors of calves in HI-STEP and HI-GRAD are indicators that calves were not ready for weaning.

Key Words: neonatal, pre-weaning, post-weaning, sucking behaviors, lie behavior

Introduction

Calf raisers often use solid feed intake to determine if a calf is ready to be weaned from milk or milk-replacer. Before 2010, many U.S. dairy heifers were fed what is now considered by nutritionists and industry a “low” plane of nutrition of milk or milk-replacer (e.g., 20% CP, 20% fat, 0.45 kg/d of DM), weaned at an average of 6 weeks, although some calf-raising specialists wean as late as 9 weeks (Hulbert and Moisá, 2016). Most U.S. dairy calf raisers begin weaning calves from milk using a variation of step-down weaning (Hulbert and Moisá, 2016), which

includes either removing a feeding or decreasing milk concentration to motivate calves to consume more of their readily available solid feed. Recently, milk replacer programs of moderate or high planes of nutrition (e.g. MOD = 25% CP, 17% fat, 0.66 kg/d of DM; HI = 25% CP, 17% fat, 1.09 kg/d of DM) have been adopted. Calf raisers anecdotally reported that calves fed with HI-milk replacer programs were not motivated to consume solid feed prior to step-down weaning. Researchers then confirmed that calves fed high MR programs consumed less solid feed prior to weaning compared to calves fed conventional programs (Terre et al. 2007, Hill et al., 2010; Ballou et al., 2013). This early programming of solid feed intake behaviors may be important to overall growth of HI-fed calves. Researchers reported improvements in average daily gain among HI-fed calves compared to calves fed a moderate-plane of nutrition, dissipated after 4 months of age. A possible way to reduce the challenges of weaning calves HI-fed milk replacer is to decrease milk replacer allowance gradually over several days (Dennis et al., 2018). Although increased post-weaning digestion and growth after weaning for HI-fed calves on 3 wk step-down period compared to HI-fed calves on a 1wk step-down was reported (Hill et al., 2016). Important behaviors related to overall growth and "weaning-readiness" were not measured or reported. Cross-sucking is cause of concern among calf raisers by the time calves are grouped after weaning. It is known that teat-fed calves express less non-nutritive oral behaviors compared to bucket-fed calves (Veissier et al., 2002). This behavior is associated with the lack of suckling rather than the ingestion of milk itself (Rushen and de Passillé, 1995). However, restrictions of milk during weaning time were reported to increase duration and frequency of non-nutritive oral behaviors (Rushen and de Passillé, 1995) even for teat-fed calves. Duration of non-nutritive oral behaviors (NNOB) reduced when calves displayed more non-nutritive sucking even after a smaller meal (Rushen and de Passillé, 1995). Nevertheless, similar but smaller effects were

observed when a non-nutritive teat was provided after a meal (Veisser et al., 2002). Perhaps an environmental enrichment device and automated collection of non-nutritive oral behaviors would give better indication of "weaning-readiness" than amount of solid-feed intake alone. Calf raisers do not typically weigh back refusals; rather, they visually assess residual feed once or twice daily. Automated collection of behaviors potentially provides semi-real time assessments of individual calf affective state at high sampling rate. Therefore, for this thesis, the author focused on two major classes of behaviors in addition to solid feed intake: non-nutritive sucking of a dummy-nipple (EED) and postural positions in stand-lie behaviors. Our laboratory and other researchers demonstrated that non-nutritive sucking of an additional nipple provides environmental enrichment for calves (Hulbert et al., 2015; Horvarth et al., 2017). In addition, Sharon et al. (2019) demonstrated that usage of an environmental enrichment device served as a sensitive indicator of "weaning readiness" among calves fed HI-milk replacer than solid feed and water intake. Automated collection of Stand-Lie behaviors has also served as indicators of calf wellbeing and comfort; calves that have a high frequency of stand-lie bouts, especially at night hours, may be in a more vigilant or frustrated affective state at weaning (Hulbert et al., 2019; Bonk et al., 2013). Usage of 3-axis accelerometer data loggers such as the Hobo Pendant G (Onset Computer Corporation, Bourne, MA) was validated for precisely recording lying behaviors in dairy cows (Ledgerwood et al., 2010), while Bonk et al. (2013) validated the same accelerometer for precise measures of lying time and bout frequency for young dairy calves. Therefore, the objective of this study was to compare solid-feed residual weights, EED, and stand-lie behaviors among calves in four different weaning strategies to predict weaning readiness.

Material and methods

Animals and facilities

This study was conducted at the Nurture Research Center nursery in southwest Ohio. All calves were under the approval of the institutional animal care and use committee; and cared accordingly to the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010). At 3 or 4 (SD) days of age, 28 Holstein bull calves were transported approximately 300 km for 3.5 h to the facility from a Fair Oaks farm in Indiana. Calves were housed in 1.2 x 2.4 m individual pens with deep wheat straw bedding inside a non-heated barn with natural ventilation and side curtains. A French-drain system managed liquid waste to reduce pen moisture and fresh wheat straw was provided as needed. Temperatures ranged from 0-33^{o}C (21^{o}C average) and the relative humidity ranged from 17-100% (77% average).

Timelines and treatments

Until weaning, all calves were fed milk replacer twice daily (0600 h and 1530 h) via nipple-buckets. Milk replacer was a formula common to all treatments. During their first 3-4 d of life, calves were fed one type of milk replacer (25% CP, 17% fat, 0.66 kg of DM). Throughout experiment, calves were fed water and one type of textured calf starter (20% CP and 37% starch; specific fatty acids were added as in Hills et al., 2011) *ad libitum*. At age d 4, calves were randomly assigned to one of 4 nutrition-weaning programs (Figure 2.1). Calves in the MOD-STEP program were fed a moderately high plane-of-nutrition (Figure 2.1; n = 7) and conventional stepdown weaning was initiated at age 43 d. For step-down method, the PM feeding was withdrawn for 3 days (i.e., weaning initiation), followed by elimination of the AM-feeding (i.e., weaning completion). All other calves were fed a high plane of milk replacer nutrition (HI; Figure 2.1). Calves in HI-STEP program (Figure 2.1; n = 7) were weaned using

conventional step-down method, which was initiated at age 39 d. At age 50 d, weaning was initiated for HI-LATE (Figure 2.1; n = 7) calves by removal of PM-feeding but they were provided 7 days of AM-feeding until weaning completion. Weaning was initiated at age 43 d by reducing both AM- and PM-feeding solids to 0.43 kg for HI-GRAD calves (Figure 2.1; n = 7); then, they were gradually weaned by reducing the solids by 25-35% increments in three more steps over next 10 days (Figure 2.1), and they were completely weaned at age 57 d.

Weaning readiness measures

At 6 d, 28 calves (n = 7 per TRT) were provided an environmental enrichment device (EED). The EED consisted a dummy nipple and bottle with sensor and logger to track in events every time calf manipulated the nipple (20 Hz sensitivity of movement; 1 Hz collection-rate, HOBO State Data Logger UX90–001M). Environmental enrichment device was provided until 1 wk after weaning. A 3-axis accelerometer (Onset Computer Corp., Bourne, MA) was attached medially to hind-leg of each calve for the same time period, logging in 60 sec intervals.

Growth and health measures

Weights were measured a day after arrival (4 d; 43 ± 1.2 kg of initial BW) and then weekly. Data for growth are reported elsewhere (Dennis et al., 2018). For monitoring of health, calves were visually observed, and fecal scores were collected daily using a 1 to 5 scale (1 – normal, 5 – watery) (modified from Kertz and Chester-Jones, 2004). Medical treatments using antibiotics were also recorded daily accordingly to a binomial scoring system of lethargy, coughing, nasal discharges, or rectal temperature (> 39^{o}C). Even though electrolytes were provided to calves with fecal scores > 2, this procedure was not counted as medical treatment.

Statistical analyses

Sampling

The automated and starter-intake data were sampled for 3 days prior to weaning initiation (Wi) (Pre-wean), first 3 days after Wi, and first 3 days after weaning completion (Wc). Prior to analyses, these data were checked for normality of residuals by evaluating Shapiro-Wilk statistic using UNIVARIATE procedure of SAS (v. 9.2, SAS Inst. Inc., Cary, NC, USA), and all data were normally distributed. From these data, a linear, mixed model with fixed effects of time, treatment, and interactions of treatment vs time was fitted and analyzed by restricted maximum likelihood ANOVA using MIXED procedure of SAS. Random effect was calf nested within treatment. Compound symmetry covariance structure for the within-subject measurement was used for all models. Pair-wise comparisons were performed 1) among treatments at each time using a sliced-effect multiple comparison approach and 2) within each treatment across time using a Tukey-Kramer adjustment to control family-wise Type-1 error. Least square means (± SEM) are reported throughout. In addition, every calf was categorized as a frequent EED-user (< 400 events per day) or a moderate EED-user (≥ 400 events per day). Categorical data and treatments were analyzed using Chi-square analysis in SAS. Treatment, time, and interaction differences of $P < 0.05$ were considered significant and $P > 0.05 < 0.10$ when biologically appropriate were considered tendencies.

Results

Performance measurements were reported by Dennis et al. (2018). Calves in MOD-STEP (76.2 kg; SEM = 1.633) treatment were lighter by 56 d (pre-weaning) when compared to HI-STEP, HI-LATE, and HI-GRAD (77.5 kg, 80 kg, and 81.2 kg respectively; SEM = 1.633).

However, on day 112 post-weaning MOD-STEP calves body weight (138.9 kg; SEM = 2.065) were heavier than HI-STEP and HI-LATE (134.7 kg and 136.9 kg; SEM = 2.065) and lighter than HI-GRAD (139.9 kg; SEM = 2.065). No growth rates improvements were observed for HI-GRAD calves, while MOD-STEP calves had similar results in body weight and structural growth as high milk replacer calves (Dennis et al., 2018).

Although there were treatment vs time differences for sampled solid-feed intake and automated behavioral data (Table 2.1; $P < 0.05$). Weaning caused calves in MOD-STEP treatment to increase (Figure 2.2; $P < 0.05$) solid-feed intake in two steps (weaning initiation and completion). Total lie-duration, left-sided lying, and number of lying bouts increased among MOD-STEP calves after weaning (Figure 2.3; $P < 0.05$). However, MOD-STEP did not change their EED-use (Figure 2.4; $P > 0.10$). Calves in MOD-STEP treatment corresponded to 14.3% of the total frequent EED-users (Table 2.2; $\chi^2 = 63.59$, $P < 0.001$). Weaning also caused calves in HI-STEP treatment to increase (Figure 2.2; $P < 0.05$) solid-feed intake in two steps (weaning initiation and completion). Total lie-duration and left-sided lying did not change throughout time (Figure 2.3; $P > 0.10$) and number of lying bouts decreased among HI-STEP calves after weaning (Figure 2.3; $P < 0.05$). However, HI-STEP calves have an increase use of their EED during weaning (Figure 2.4; $P < 0.05$). Additionally, HI-STEP calves corresponded to 42.9% of the total frequent EED-users, which was higher than expected (Table 2.2; $\chi^2 = 63.59$, $P < 0.001$). For HI-LATE calves, weaning completion increased solid-feed intake yet there was no difference between before and after weaning (Figure 2.2; $P > 0.10$). Total lie-duration, left-sided lying and lying bouts did not change during and after weaning (Figure 2.3; $P > 0.10$). Calves on HI-LATE treatment did not had a change their EED-use (Figure 2.4; $P > 0.10$). Percentage of frequent EED-users was lower than expected (Table 2.2; $\chi^2 = 63.59$, $P < 0.001$). For calves in

HI-GRAD treatment weaning completion caused an increase solid-feed intake (Figure 2.2; $P < 0.05$). However, there was no differences between before and after weaning (Figure 2.2; $P > 0.10$). Total lie-duration, left-side lying and lying bouts did not changed during and after weaning (Figure 2.3; $P > 0.10$). However, HI-GRAD did not change their EED-usage throughout weaning process (Figure 2.4; $P > 0.10$), 33.3% of calves in this treatment were frequent EED-users, which was higher than expected (Table 2.2; $\chi^2 = 63.59$, $P < 0.001$).

Discussion

Calves in MOD-STEP increased solid-fed intake gradually and according to milk replacer withholdings. This could be attribute to the fact that calves were hungrier during milk replacer withdrawals and needed solid feed to complement their nourishment, similarly to results from previous studies (Jensen, 2006; Borderas et al., 2009). However, calves in HI-STEP treatment had a gradual increase in solid-feed intake but much lower than compared to MOD-STEP calves. While calves in HI-LATE and HI-GRAD treatments had a sudden rise in solid-feed intake after milk replacer was completely withdrawn. Similar results were reported by Borderas et al. (2009), indicating that HI-fed calves take longer time to catch up with moderate-fed calves solid feed intake. In virtue of this, later solid-feed intake rise similar structural growth was found between moderate- and HI-fed calves (Dennis et al., 2018). Researchers reported that activity was higher than expected in calves fed moderated milk replacer nutrition (De Paula Vieira et al., 2008). Similarly, in our study MOD-STEP calves were more active before and during weaning, although time spent lying and the number of lying bouts were significantly greater after weaning completion. Time spent lying on left-side was greater for MOD-STEP calves after weaning compared to the other treatments. Tough, the amount of time spent lying on

the left was about half of the total time lying. Calves on HI-STEP treatment spent significantly greater time lying and had a higher number of lying bouts, particularly during weaning compared to other treatments. While HI-STEP calves did not have a significant change in their time spent lying or lying on their left, the number of lying bouts significantly decreased during and after weaning completion. This is an indication that even though HI-STEP calves did not decrease their time lying, their time napping was lower than 120 sec of duration and thus was not considered a bout. That may be a cue that those calves were more active than we previously assumed just looking into lying time. Additionally, HI-GRAD calves had a slightly, but not significant, reduction in lying time during weaning, but no changes were observed in lying on their left or on number of lying bouts. In a study looking for standing time variances in less than 2 mo of age Holstein calves in different periods of the year, researchers found out that calves averaged 300 min per day standing but no differences were found between time periods (Hill et al., 2013). Our study had similar results for treatments MOD-STEP and HI-LATE: standing time averaged 310 and 314 min per day, respectively. Treatments HI-STEP and HI-GRAD spent more time standing when compared to previous studies, which could be an indication that those calves were less comfortable during weaning progress. Our Environmental Enrichment Device (EED) provided a way for calves to manipulate and suck a pacifier performing non-nutritive oral behaviors (Metz, 1984; Jung and Lidfors, 2001). A non-nutritive sucking device such as a pacifier was already reported to induce a calmer state in rats and human infants (Blass, 1994), although in this study a high usage of EED was observed in HI-STEP and HI-GRAD calves around weaning. These two treatments also had calves spending less time lying down, indicating restless behaviors. These calves still seem motivated to suckle in comparison to the other two groups, and for that reason they are not ready to be weaned. To wean these calves could become

a concern if sucking behaviors are still in place. When commingled they could likely start suckling on each other increasing pathogen spread and infections (Veissier et al., 2002). In contrast to other researchers' findings, calves fed moderate milk replacer did not use EED as frequently as we expected. In addition, increased stereotypies behaviors were reported in sows (i.e., bar chewing), lactating dairy cows, and broiler breeders and were associated with restricted feeding systems (de Jong et al., 2002; Redbo et al., 1996; Terlouw et al, 1991). An increase in vocalization by calves was also reported in moderate milk replacer systems (Thomas et al., 2001) but these observations were not made during this study. Further research using video footage can be implemented in the future to address vocalizations.

Conclusions

In conclusion, behavioral measures in this study could be used as indicator of weaning readiness. Calves that are still motivated to suckled are not ready to be weaned or commingled. Increased activity after weaning could likely be an indication of discomfort during the weaning process, where they spend less time lying and use EED more frequently. High plane milk replacer nutrition did not increase the weaning readiness among calves in HI-STEP and HI-GRAD. However, calves in HI-STEP and MOD-STEP had similar results, a longer weaning protocol seems to mitigate discomfort and improve weaning readiness. An environmental enrichment device and lying behaviors can be used by calf raisers as indication that calves are ready or not to be weaned. These tools can also help in decision making around weaning and commingling time.

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Tables

Table 2.1. Solid-feed intake and automated behaviors of calves fed four different milk replacer (MR) programs during three periods relative to weaning initiation sampled and averaged (72 h before weaning, just after initiation wening, and just after weaning completion).

	Treatment[1]					Time[2] relative to initiation of weaning				*P* - values		
	MOD-STEP	HI-STEP	HI-LATE	HI-GRAD	SEM[3]	Pre	During	After	SEM[3]	TRT	Time	TRT x Time
Number of calves	7	7	7	7	—	28	28	28	—	—	—	—
Solid-feed g/d	1077[a]	530[b]	1046[a]	937[a]	86.60	366[a]	659[b]	1668[c]	0.04	<0.001	<0.001	<0.001
Lie position, min/d	1130[a]	1079[a]	1126[a]	1072[a]	18.70	1121[a]	1078[b]	1106[ab]	12.92	0.067	0.017	0.001
Lie Left[4], %	591.2[a]	552.7[a]	571.0[a]	534.9[a]	20.64	563.3[a]	545.1[a]	578.8[a]	14.50	0.261	0.156	0.005
Lying bouts[5], no./d	24.5[a]	23.1[a]	25.1[a]	23.9[a]	1.66	25.3[a]	23.0[b]	24[ab]	22.40	0.845	0.036	<0.001
EED, sec/d	152.6[a]	306.6[ab]	101.7[ac]	253[abc]	21.80	200.8[a]	224.8[a]	184.8[a]	29.70	0.036	0.101	<0.001
EED bouts[6], no./d	3.2[a]	11.1[ab]	7.3[a]	22.0[b]	3.55	7.3[a]	14.8[b]	10.6[a]	1.94	0.006	<0.001	<0.001

[a-d-c]LS-means differ ($P < 0.05$; Tukey-Kramer adjustment); [1]MOD-STEP= 0.66 kg of DM/d for the first 39 d of treatment and 0.33 kg only AM for 3 d; HI-STEP = 0.87 kg of DM/d for 4 d, 1.09 kg for 31 d, and 0.54 kg only AM for 7 d; HI-LATE = 0.87 kg of DM/d for 4 d, 1.09 kg for 42 d, and 0.54 kg only AM for 7 d; HI-GRAD = 0.87 kg of DM/d for 4 d, 1.09 kg for 35 d, and 0.87 kg for 4 d, 0.66 kg for 4 d, 0.44 kg for 3 d, and 0.22 kg only AM for 3d; [2]72 h of continuous data were sub-sampled to represent each time period, Pre: Before Weaning, During: During weaning, After: After weaning; [3]Largest SEM; [4]While in the lie-position, the time in min each d calf leaned to the left. [5]The number of times per day calves lie for longer than 120 sec. [6]The number of times calf moved from not touching to touching the EED for at least 2 sec with 1 sec interval.

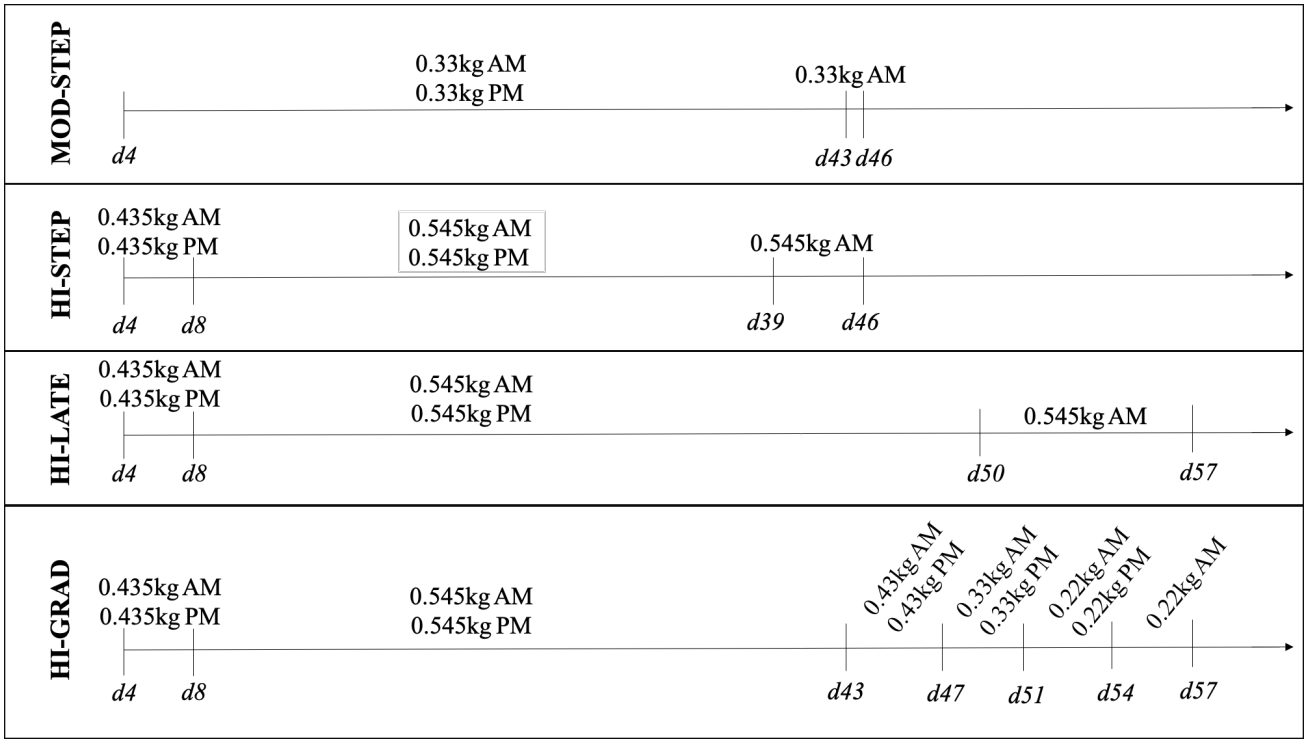
Table 2.2. Percentage of normal (< 400 sec of EED usage per day) and frequent (> 400 sec of EED usage per day) EED users for each treatment during the 72 h pre-, during- and after-weaning. Chi-square analysis. $\chi^2 = 63.59$, $P < 0.001$.

	Normal[1]				Frequent[2]			
Treatment	**n[3]**	**%[4]**	**Expected[5]**	**Residuals**	**n[3]**	**%[4]**	**Expected[5]**	**Residuals**
MOD-STEP	6	85.7	90	2.35	1	14.3	10	-2.35
HI-STEP	4	57.1	90	-5.66	3	42.9	10	5.66
HI-LATE	7	100.0	90	6.22	0	0.0	10	-6.22
HI-GRAD	5	66.7	90	-2.90	2	33.3	10	2.90

[1]Normal EED users (< 400 sec of EED usage per day); [2]Frequent EED users (> 400 sec of EED usage per day); [3]Total number of calves that were categorized as normal or frequent EED users. [4]Percentage of calves in each treatment that were categorized as normal or frequent EED users. [5]Expected percentage of calves for each group (normal and frequent) according to Chi-square analysis.

Figures

Figure 2.1. Milk replacer (MR) time line with the following treatments: (1) 0.66 kg of DM/d during the first 39 d of treatment and 0.33 kg only a.m. for 3 d (**MOD-STEP**); (2) 0.87 kg of DM/d for 4 d, 1.09 kg for 31 d, and 0.54 kg only AM for 7 d (**HI-STEP**); (3) 0.87 kg of DM/d for 4 d, 1.09 kg for 42 d, and 0.54 kg only AM for 7 d (**HI-LATE**); and (4) 0.87 kg of DM/d for 4 d, 1.09 kg for 35 d, and 0.87 kg for 4 d, 0.66 kg for 4 d, 0.44 kg for 3 d, and 0.22 kg only AM for 3d (**HI-GRAD**).



d = days of age; AM = morning feeding; PM = evening feeding;

Figure 2.2. Box-plot (center line = mean, error bars = standard deviation, and points = outliers) of grain intake from MOD-STEP (0.66 kg of DM/d for the first 39 d of treatment and 0.33 kg only AM for 3 d), HI-STEP (0.87 kg of DM/d for 4 d, 1.09 kg for 31 d, and 0.54 kg only AM for 7 d), HI-LATE (0.87 kg of DM/d for 4 d, 1.09 kg for 42 d, and 0.54 kg only AM for 7 d), HI-GRAD (0.87 kg of DM/d for 4 d, 1.09 kg for 35 d, and 0.87 kg for 4 d, 0.66 kg for 4 d, 0.44 kg for 3 d, and 0.22 kg only AM for 3d) represented by 72 h of continuous data sub-sampled to represent each time period. Pre-weaning, During-weaning, and After-weaning. *P* values for TRT, TIME, TRT*TIME were <0.01. [a-e] LS Means differ ($P < 0.05$; Tukey-Kramer adjustment). For each plot, the box upper and lower limits represent the IQR (1º and 3º quartiles), the bold line within the box represents the median, the whiskers delimit the range and the circles represent the outliers.

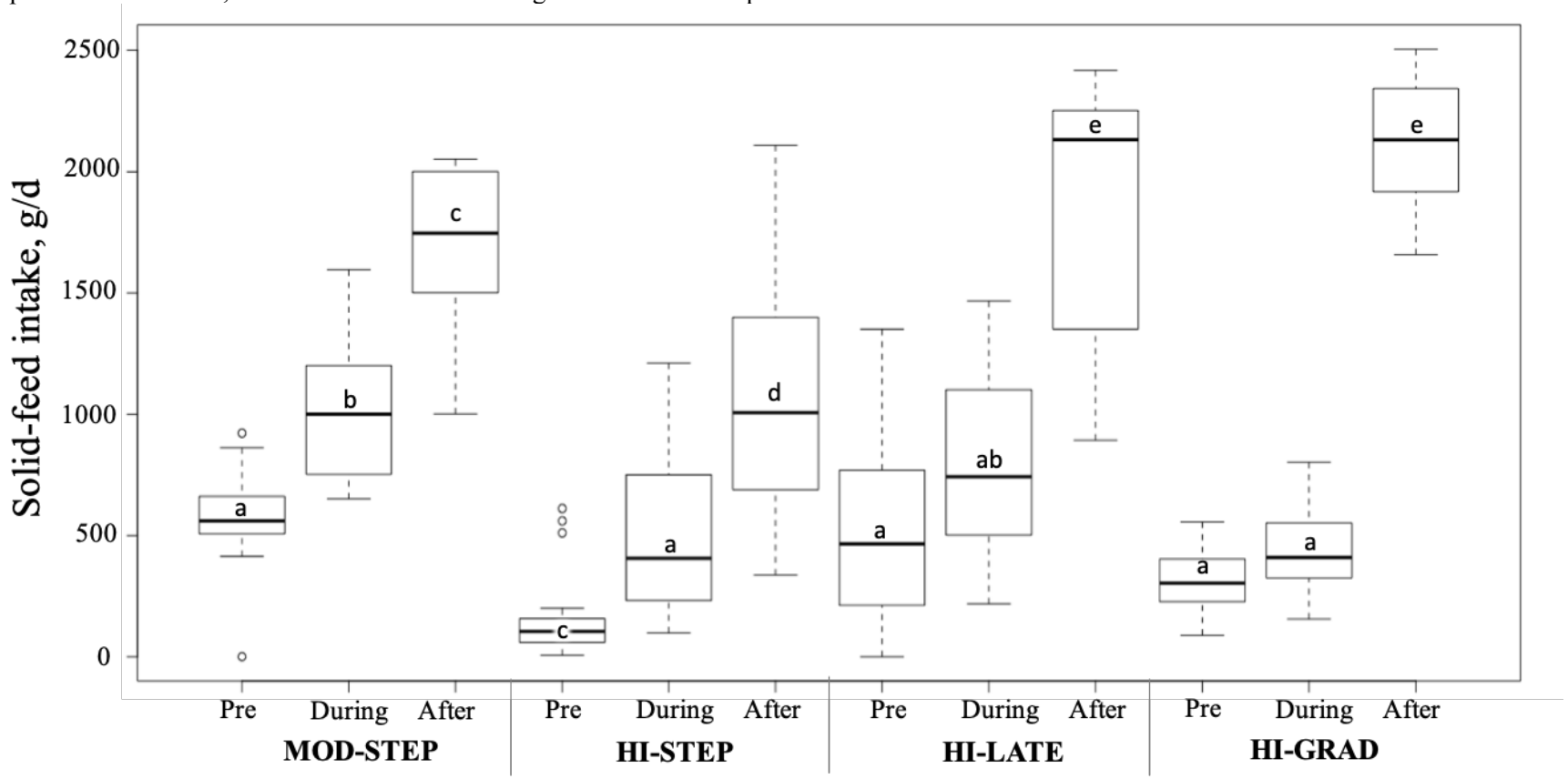


Figure 2.3. Estimates (± SE) of (A) time spent in lie position, (B) time spent in lie position leaned to left, (C) number of times in lie position < 2 min, by treatment (MOD-STEP= 0.66 kg of DM/d for the first 39 d of treatment and 0.33 kg only AM for 3 d; HI-STEP = 0.87 kg of DM/d for 4 d, 1.09 kg for 31 d, and 0.54 kg only AM for 7 d; HI-LATE = 0.87 kg of DM/d for 4 d, 1.09 kg for 42 d, and 0.54 kg only AM for 7 d; HI-GRAD = 0.87 kg of DM/d for 4 d, 1.09 kg for 35 d, and 0.87 kg for 4 d, 0.66 kg for 4 d, 0.44 kg for 3 d, and 0.22 kg only AM for 3 d) and represented by 72 h of continuous data were sub-sampled to represent each time period. Pre-weaning, During-weaning and After-weaning. * Treatment by time LS means differ ($P < 0.05$; Tukey-Kramer adjustment). ✝ Within treatment LS means differ ($P < 0.05$; Tukey-Kramer adjustment).

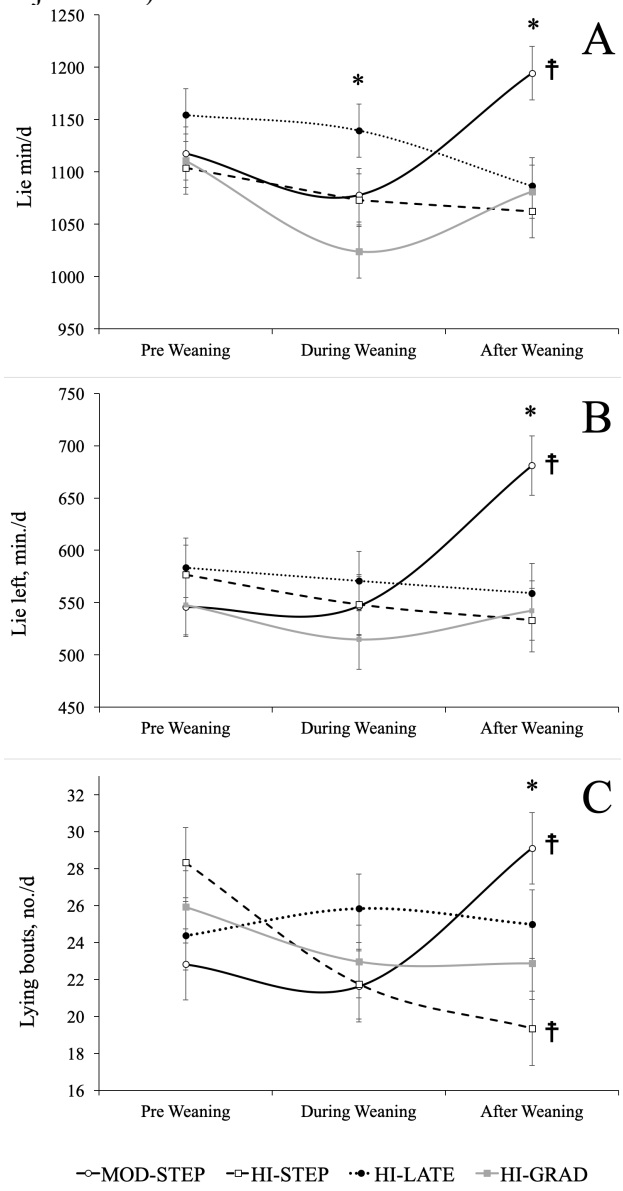
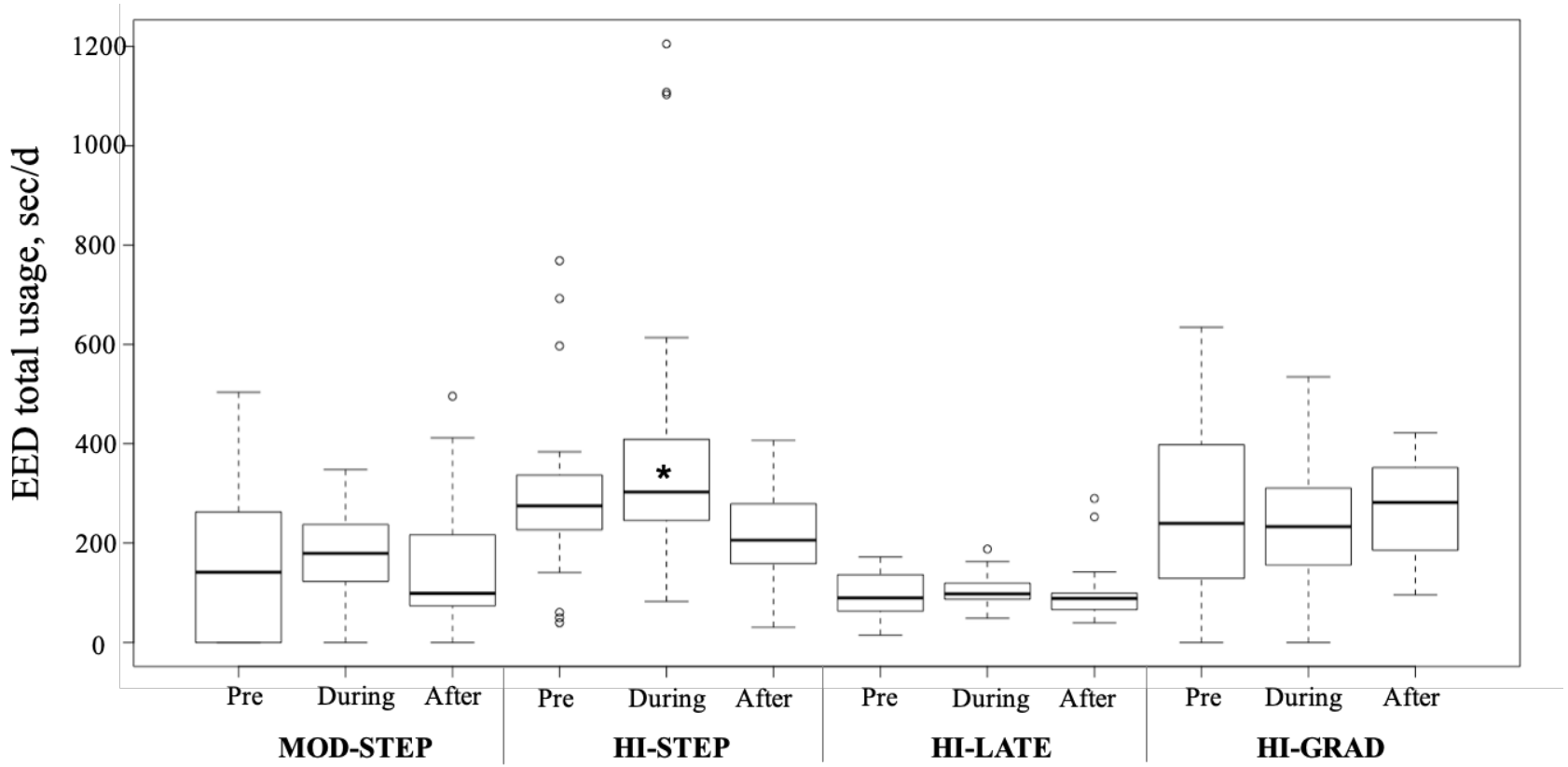


Figure 2.4. Box-plot (center line = mean, error bars = standard deviation, and points = outliers) of EED total usage from MOD-STEP (0.66 kg of DM/d for the first 39 d of treatment and 0.33 kg only AM for 3 d), HI-STEP (0.87 kg of DM/d for 4 d, 1.09 kg for 31 d, and 0.54 kg only AM for 7 d), HI-LATE (0.87 kg of DM/d for 4 d, 1.09 kg for 42 d, and 0.54 kg only AM for 7 d), HI-GRAD (0.87 kg of DM/d for 4 d, 1.09 kg for 35 d, and 0.87 kg for 4 d, 0.66 kg for 4 d, 0.44 kg for 3 d, and 0.22 kg only AM for 3d) represented by 72 h of continuous data sub-sampled to represent each time period. Pre-weaning, During-weaning, and After-weaning. *P* values for TRT, TRT*TIME were <0.05. * LS Means differ ($P < 0.05$; Tukey-Kramer adjustment). For each plot, the box upper and lower limits represent the IQR (1º and 3º quartiles), the bold line within the box represents the median, the whiskers delimit the range and the circles represent the outliers.



Chapter 3 - Association among neonatal beef calf behaviors, dam blood parameters, and immune-status of cows and calves

Abstract

Calf suckling behaviors after birth have a direct effect on risk for morbidity and mortality, yet measures of hematology, stress, and immunity are typically used as biomarkers for health. Therefore, the overall objective was to detect if calf innate behaviors and maternal blood measures can serve as biomarkers for passive transfer and replace invasive measurements techniques. For this observational study, two-year-old Angus-cross heifers (n = 59; South Dakota, USA, March 2018) were moved to a maternity pasture. Before moving heifer body weight was measured, and a total of 8-mL of whole blood (EDTA and Heparin) was collected via jugular venipuncture. Three trained observers (inter-observer reliability > 95%) monitored calving progression in 24-h shifts. Times were collected for each calf's: birth (calf on the ground); stand (all four limbs upright for >5 seconds); first-suckle (mouth contact with any teat); and each teat during the first 24 h of life. Relative to birthing time, latency to stand, first-suckle, and to suckle on all four teats were calculated. After the 24 h observation period, calf bodyweights were measured and a total of 8 mL of whole blood (EDTA and Heparin) via jugular venipuncture were collected. All EDTA-blood samples were used to measure complete blood counts (CBC; Idexx Procyte, Westbrook, ME, USA). Plasma was analyzed for: Immunoglobulins: G1 and M (Bethyl Laboratories Inc., Montgomery, TX), cortisol (DetectX®; Arbor Assays, Ann Arbor, MI, USA), and haptoglobin concentrations (Hp). Data were analyzed for descriptive statistics, ANOVA using the GLM analyses, a t-test analyses and Pearson's

Correlation in SAS (v. 9.2, SAS Inst. Inc., Cary, NC, USA). Mean differences were reported for two groups of calves: failed and succeed passive immune transfer, using a threshold of 10 g/L of plasma IgG. Seventeen percent of the calves failed to acquire passive immune transfer from maternal colostrum. Same calves took a longer time to stand up ($P < 0.001$), a longer time to first suckle ($P < 0.05$), and a longer time to suckle least teat ($P < 0.05$). As expected, calf total plasma protein, IgG, and IgM concentration were higher for Succeed calves (respectively: $P < 0.001$; $P = 0.052$; and $P = 0.052$). When comparing heifer and calf groups, hematocrit, hemoglobin, MCV, MCH, MCHC, and lymphocytes mean values were higher in heifers ($P < 0.001$), while total erythrocyte counts, and neutrophils were higher in calves ($P < 0.001$). Plasma bactericide activity and haptoglobin concentrations were higher in heifers, which may be due to parturition inflammation. Using correlations, calf total plasma protein (TPP) increased when latency between birth to stand or birth to first suckle decrease (respectively: r = -0.45, $P < 0.01$; r = -0.24 $P = 0.08$). Positive correlations were founded between calf TPP, IgG1 and IgM concentrations (r = 0.52, $P < 0.01$). Gestation length tended to cause increased neonatal calf cortisol concentrations (r= 0.24, P =0.07). Calf precocious behaviors can be used by producers as biomarkers to measure calf successful passive immune transfer and replace invasive techniques.

Introduction

The hours after birth are a critical window for behavioral development in calves (Hulbert and Moisá, 2016). Some calves can have innate precocious behaviors and take less time to obtain their first meal than calves with a delayed development. The ruminant placenta is impermeable to antibodies (Chucri et al., 2010) and nonetheless, colostrum is important to be ingested immediately after birth. This first meal is particularly important for bovine neonates because they

need to ingest colostrum within 24 h of birth in order to gain passive immunity from their dams (Stott et al., 1979b; Gay, 1984; Godden, 2008; Murray and Leslie, 2013). Colostrum will also help the development of non-specific immunity such as mucosal barriers that will protect neonatal calf from pathogens without an inflammatory response (Hulbert and Moisá, 2016; Kelly and Coutts, 2000).

When time for the calf to stand and start suckling (i.e. latency-to-suckle) is greater, then less immunoglobulins and nutrients are absorbed from colostrum. Researchers reported that closure of intestinal permeability occurs approximately 24 h after birth (Besser and Gay, 1994; Pakkanen and Aalto, 1997; Moore et al., 2005) due to maturation of intestinal epithelial cells (Stott et al., 1979b). The critical window for colostrum ingestion may even be shorter than 24 h, Stott et al. (1979a) reported that Holstein-Friesian calves fed colostrum after 12 h post-calving, had failure of passive transfer (FTP) due to intestinal permeability closure. Based on gut closure timeline, other researcher (Gay, 1984) recommended feeding colostrum as soon as 2 h after birth.

Influence of maternal state, even among apparently normal births is well studied when related to nutrition and body scores. Lower vitality and lower passive immune transfer were reported in calves born from heifers with lower body conditions (Odde, 1996; Carstens et al., 1987). Protein restrictions for heifers during pre-partum were shown to reduce thermoneutral metabolism in neonatal calves (Carsterns et al., 1987). This also reduces calves' thermogenesis ability to respond to cold stress after parturition (Smith and Carsterns, 2005). In abnormal parturition researchers reported increase in cortisol concentrations in dams and calves (e.g. dystocia; Vannuchi et al., 2015). In addition, dystocia can increase the risk for calves to develop respiratory acidosis which later on will affect passive immune transfer (Quigley and Drewry, 1998). Besides nutritional and birth difficulties, maternal status can also influence calf

hematological and biochemical status (Shil et al., 2012). However, cow and calf comparisons are not commonly reported.

Over half of dairy producers will hand feed by bottle or gavage calves to mitigate passive immune transfer critical window (NAHMS, 2014). In contrast, beef production is more extensive, and calves are allowed to seek and suckle before a human intervenes (Von Keyserlingk and Weary, 2007; Besser and Gay, 1994). Calf raisers have the option of measuring passive transfer using spectrophotometers to quantify the total amount of protein present in calves' blood (Deelen et al., 2014; Elsohaby et al., 2015; Stott et al., 1979). Calf total serum or plasma protein were reported to be highly correlated with total immunoglobulin G (IgG; r = 0.87; Morrill et al., 2013). Other antibody-subtypes are directly measured (e.g. IgG1 and IgM), but methods for data collection are not rapid, and therefore, by the time the calf raiser receives the information, there may not be an opportunity for intervention. Therefore, the overall objective was to detect if calf innate behaviors and maternal blood measures can serve as biomarkers for passive immune transfer and replace invasive techniques.

Material and methods

Animals and facilities

This study was conducted in March and April 2018 at a cow-calf operation ranch near Pollock, north central South Dakota. Angus-cross heifers (n = 59; age 2 years) were housed in approximately 0.4 km^2 maternity pasture. Heifers were provided a nutritional ration one time a day and water *ad-libitum*. In mild weather conditions, heifers were allowed to give birth in the maternity pasture. Within the maternity pasture, a calf-nesting area was set up and consisted of wind shield panels and 20 cm-deep straw bedding at maternity pasture. Small entry ways only

allowed the calves to enter calf-nesting area preventing peripartum heifers from stepping on nesting calves. When weather conditions became harsh, animals were moved into a maternity barn. Maternity barn was approximately 4047 m^2 divided in twelve maternity pens, a maternity chute, an area for food and bedding storage, and an area with water source. Maternity pens were bedded with straw 10 cm deep. Daily, soiled and dirty bedding were removed, and new straw was added. Heifers were allowed inside only for 24 h to assure bond with their calf; afterwards they were moved out into the maternity pasture. Wind shield panels and bedding were also provided as a shelter for heifers close to the calf bedding area.

Behavior collection and live observations

Three trained observers (inter-observer reliability > 95%) monitored calving progression in 24-h shifts. Times were collected for each calf's: birth (calf on the ground); stand (all four limbs upright for >5 seconds); first-suckle (mouth contact with any teat); and each teat during the first 24 h of life. Relative to birthing time, latency to stand, first-suckle, and latency to suckle on all four teats were calculated.

Blood collection and body weight

Heifers

Heifer blood was collected before moving into maternity pasture. A hydraulic squeeze chute was used to restrain heifers during collections. A total of 8 mL of blood was drawn from jugular venipuncture using a vacutainer system (20-gauge x 1 ½ inch) into two tubes with anticoagulants (EDTA and Heparin). During blood collections pre-calving heifer body weights were recorded using a calibrated scale from a hydraulic cattle chute.

Calves

Calf blood was also collected via venipuncture but 24-48 h after birth. Calves were gently handled and restrained in left side decubitus for collection. A total of 8 mL of blood was drawn from jugular venipuncture using a vacutainer system (22-gauge x 1 inch) into two tubes with anticoagulants (EDTA and Heparin). Calf birth weight was recorded 24-48 hours after birth using a mobile scale.

Blood analyses

A field laboratory was set up at the ranch shop to process and do a pre-analysis of blood. All EDTA-blood samples were used to measure complete blood counts (CBC; Idexx Procyte, Westbrook, ME, USA).

Immune measures

Heparin-blood samples were immediately centrifuged at 230 rfc for 15 min. Plasma was harvested and frozen at -20°C for further analysis. At Kansas State University animal physiology lab, plasma samples were unfrozen overnight in a refrigerator and analyzed for immunoglobulins (IgG1 and IgM), cortisol, haptoglobin concentrations (Hp), plasma bactericide assay (PB), and total plasma protein (TPP). For all assays, samples were randomly assigned to each assay protocol. Immunoglobulins measures, IgG1 and IgM, were analyzed using a commercially available ELISA kit (Bethyl Laboratories Inc., Montgomery, TX) with suggested plasma dilutions. Optical density was estimated at 450 nm in a microplate reader and concentrations were calculated using an app (elisaanalysis.com). Intra- and inter-assay coefficient of variations were respectively 3.82% and 14.8% for IgG1, and 5.21% and 0.43% for IgM. A refractometer was used to measure refractive index (BRIX) of total plasma protein (TPP).

Cortisol measurements were calculated using a commercially available ELISA kit (DetectX®; Arbor Assays, Ann Arbor, MI, USA) in 15 µl of plasma. Optical density was estimated at 450 nm in a microplate reader and concentrations were calculated using an app (elisanalysis.com). Intra- and inter-assay coefficient of variation were respectively 3.90% and 4.12%.

Acute-phase protein haptoglobin (Hp) concentrations were measured based on colorimetric method using peroxidase activity previously described (Cooke and Arthington, 2013). High concentration Hp samples were used to prepare standards and curved with serial dilutions of 1:1 ratio. In a borosilicate tube (16 x 100 mm), plasma samples (10 µl) and 7.5 ml of O-dianisidine solution were incubated for 45 min at 37.5°C. Hemoglobin solution (25 µl) was then added followed by more 45 min of incubation at same temperature. Hydrogen peroxide solution (100 µl) was added after incubation and samples were incubated at room temperature for 1 h. For optical density measurements, samples were pipetted into a 96 well-plate and read at 450 nm in a microplate reader. Intra- and inter-assay coefficient of variation were respectively 2.23% and 0.07%.

Adapted methods for measurements of plasma bactericidal activity against a live culture of bacteria were based on previous described protocol (Crokaert et al., 1988). A live *E. coli* (8739®) culture was incubated with plasma sample at 1:20 ratio for 10 min. After incubation, samples were vortexed and 50 µl were cultured over tryptic soy agar plates (Difco™ Microbial Content Test Agar, Becton Dickinson Company, Sparks, MD) in duplicates. Control samples were prepared only with RPMI and live *E. coli*. Samples and control plates were incubated for 24 h at 37.5°C and the number of colony-forming units (CFU) were estimated. Percentage of CFUs eliminated by plasma were calculated using control means.

Statistical analyses

To perform the analyses, total plasma protein was converted to IgG using regression equation reported by Morril et al. 2013. The following equation was used: 9.12846x-59.2122. Failure of passive transfer was defined as plasma IgG < 10 g/L. After conversion, data were analyzed for descriptive statistics, ANOVA using GLM analyses, mean differences using a *t*-test and, Pearson's Correlation. Repeated data were tested for normality of residuals by evaluating Shapiro-Wilk statistic using UNIVARIATE procedure of SAS (v. 9.2, SAS Inst. Inc., Cary, NC, USA). Data that were not normally distributed were log- or arcsine square root-transformed before *t*-test and GLM model analysis. Comparisons were performed between two groups of calves: calves that failed (Failed calves) or succeeded (Succeed calves) in acquiring passive immune transfer from maternal colostrum using a Tukey-Kramer adjustment. Least squares mean (± SEM) are reported throughout. Differences of $P < 0.05$ were considered significant and when biologically appropriated $P > 0.05$ <0.10 were considered tendencies. A *t*-test was used to compared group means for hematology and immunology data. Animals were separated in two groups: heifers and calves. Mean group differences (± SE) are reported using a Cochran adjustment for unequal means. Differences of $P < 0.05$ were considered significant. Correlations between calf immune measures, calf behaviors, cow immune status, and gestation length were performed using Pearson's correlation for each variable. Differences of $P < 0.05$ were considered significant and when biologically appropriated $P > 0.05$ <0.10 were considered tendencies.

Results and discussion

Descriptive information

The first few hours after birth are extremely important for neonatal calves' health. During this time, calves will need to acquire the first meal through their innate behaviors. Importance of colostrum intake in domestic animals is well discussed (Besser et al., 1991). This intake is essential for ruminants due to their synephiteliochorial placenta that is impermeable to antibodies (Stott et al., 1979b, Chucri et al., 2010). Failure of passive transfer increases morbidity and mortality among dairy and beef calves (Weaver et al., 2000). To ensure successful passive transfer dairy calf raisers routinely hand-fed calves (USDA, 2014). Although, methods to measure immunoglobulins in calves' blood are invasive (i.e. blood collection) and time consuming, which can delay intervention. Maternal influences on calves' health are mostly reported throughout pre-partum nutritional studies or abnormal parturitions (Odde, 1996, Laster and Gregory, 1973). Measurements of maternal blood parameters on calves' health and behaviors are not commonly reported. Therefore, the overall objective of this study was to determine if calf innate behaviors and maternal blood measures can replace invasive techniques of passive immune transfer by identifying FPT calves, comparing maternal and offspring blood parameters, and correlating behavioral and blood measures from cows and calves.

The primarily goal was to observe innate neonatal calf behaviors outdoors, however there were several days of severe weather (Figure 3.1), with high wind speed, cold temperatures, snow, and freezing fog. Therefore, thirteen heifers gave birth outdoors and then were moved indoors, all data were collected for these calves. However, five heifers delivered outdoors before observations were collected, their observational data were removed from the analyses. Remaining forty-one heifers were moved to the indoor barn as soon as the first stage of

parturition was observed (i.e. fetal membranes with mucous, tail upright). The covariate of birthplace (indoor vs. outdoor) was examined for each variable and there was no effect ($P > 0.10$) on these variables.

Calf behaviors and immune status differences

Measures of inflammation and immunology, such as haptoglobin, cortisol, and plasma bactericide did not differ among calves that were successful or failed to acquire passive immune transfer (Table 3.1; $P > 0.10$). Using a passive immune transfer threshold of 10 g/L of IgG (McGuirk and Collins, 2004; Morril et al., 2013; Deelen et al., 2014; Elsohaby et al., 2015), 17% of calves failed to acquire passive immune transfer from maternal colostrum. As expected total plasma protein (TPP) was higher for Succeed calves (Table 3.1; $P < 0.001$). Immunoglobulins G1 and M tended to be higher for Succeed calves than Failed calves (Table 3.1; $P = 0.052$). To support the previous findings, a positive correlation was established between calf percentage of total plasma protein and calf IgG1 (Table 3.3; r = 0.52, $P < 0.01$). Morrill et al. (2013) reported an even higher correlation (r = 0.87) between total serum protein from 1 d year-old calf and IgG concentrations. This difference may be due to fact that in our study a correlation was made between TPP and IgG1 measured through an ELISA assay, while Morrill et al. 2013 correlated TPP to IgG using radial immunodiffusion. Calf plasma IgM concentrations also correlated positively with TPP, although with a much stronger correlation than IgG1 (Table 3.3; r = 0.89, $P < 0.01$). On average Failed calves took almost 69 min longer to stand up than calves that had a successful passive immune transfer (Figure 3.2; $P < 0.001$). Following the same pattern, Failed calves took longer to start suckling and longer to reach last teat suckled (Figure 3.2; $P < 0.05$). According to previous research, a progressive decrease in immunoglobulins occurs when age increases due to maturing of intestinal epithelial cells (Stott et al., 1979b). In support of the

previous statement, a negative correlation tendency was found between latency to first suckle and TPP. In that way calves that took longer to first suckle tended to have lower TPP (Table 3.3; r = -0.24; $P = 0.08$). Succeed calves' latency to stand and to first suckle were almost 30 min less than previous reported in Holstein calves (Houwing et al., 1990). Although the overall calves in this study took less than 3 hours to stand, some thoughts need to be given to the amount of colostrum intake for each individual calf. The amount of time that each calf spent suckling was not recorded in this study. Therefore, some calves may have had lower intake of colostrum than others by spending less time suckling. Previous reports showed that calves with lower vitality will take longer to stand up and probably suckle with less vigor ingesting less colostrum (Hulbert and Moisá, 2016, Kelly and Coutts, 2000). In addition, besides heifers IgG1 concentrations no other differences were found regarding heifers' immune status and FPT calves (Table 3.1; $P > 0.10$). Calves born from heifers with higher IgG1 concentrations tended to successfully acquire passive immune transfer from maternal colostrum (Table 3.1; $P = 0.09$). Previous literature reported that during pre-partum, immunoglobulins will be transported from blood to colostrum (Besser and Gay, 1994; Herr et al., 2011). That way we could assume that high IgG1 heifers also had high IgG1 concentrations in colostrum, which will influence their offspring passive immune transfer.

Differential hematological parameters between heifers and calves.

In this study percentage of eosinophils did not differ among heifers and calves (Table 3.2; $P > 0.05$). Hematocrit, hemoglobin, MCV, MHC, MCHC, lymphocytes, and monocytes were higher in heifers than calves (Table 3.2; $P < 0.001$), while TEC, reticulocytes, platelets, basophils, and neutrophils were higher in calves (Table 3.2; $P < 0.01$). Hematological parameters can indicate animals' physiology, welfare, and immune status (Shil et al., 2012). These measures

can be helpful to diagnose hematological disorders and systemic diseases (Roland et al., 2014). However, hematology parameters are age related in many species, including bovine (Brun-Hansen et al., 2006). Similarly, with our findings, researchers reported higher TEC and lower MCV, MCHC, and lymphocytes in calves when compared adult cattle (Shil et al., 2012; Brun-Hansen et al., 2006). According to Shil et al. (2012), calves have higher hemoglobin blood concentrations than cows, possibly due to more erythropoiesis, which is the development of red blood cells. Hemoglobin outcomes for this study were higher for heifers when compared to calves (Table 3.2; $P < 0.001$). In addition, bovine species were reported to have higher neutrophils and basophils during early life, which later decreases with age and the consequent maturation of their immune system (Roland et al., 2014; Rossi et al, 1979). In calves, 6 to 8 weeks are needed for lymphocytes to reach 80% of total leukocytes circulation (Brun-Hansen, 2006; Barrigton and Parish, 2001). However, the higher neutrophil concentration in neonatal calves was already addressed by previous researchers. According to Banks (1982) neutrophils are the main fetal non-specific defense. Other researchers also reported that the larger number of neutrophils in neonatal calves can be explained by the fact that their immune system relays mostly in non-specific defenses and maternal antibodies (Rossi et al., 1979). In swine and rodent species, researchers reported that leukocytes are transported from dams' colostrum to neonates' blood (Sheldrake and Husband, 1985; Williams, 1993). Despite of the fact that heifers had fewer neutrophils numbers than their offspring, dam neutrophils and basophils were (Table 3.3; $P \leq 0.05$) positively correlated with calf neutrophils ($r = 0.26$) and basophils ($r = 0.38$). The main hematological reference intervals used for cattle health are based on dairy herds (George et al., 2010), however, a reference range from a healthy animal group in similar environmental and physiological conditions should be more appropriated to diagnose hematological disorders

(Roland et al., 2014). The hematological values from Angus-cross heifers and calves describe in this study (Table 3.2) were inside the reference range previously reported by Schalm (1965) and outside the reference range reported by George et al, 2010. Since our data were collected from apparent healthy animals we can conclude that our values could be used as reference for pre-parturient Angus-cross heifers and neonatal calves in upper Midwest, United States. Thus, hematological values can be used to access animal health, different values between adult cattle and calves, their breed, and location should be taken into consideration.

Differential immunological parameters between heifers and calves

Cortisol concentrations did not differ among heifers and calves (Table 3.2; $P > 0.05$) endorsing that even eutocia is a stressful event for both dam and calf (Vannucchi et al., 2015). In contrast to previous researches, there were no correlations between dam-cortisol and calf-cortisol concentrations (Table 3.3; $P > 0.05$). Although our findings of calves' cortisol concentrations were much lower at 24 h then in Holstein calves at the same age (Uetake K., 2014). In addition, calf cortisol concentrations tended to increase with gestation length (Table 3.3; $r = 0.24$, $P = 0.07$), perhaps implying that a higher development and activity of neuroendocrine system occurs in longer-gestation-length calves. In humans, no correlations were found between gestation length and maternal cortisol (Buss et al., 2009), but an increase in maternal stress was reported to risen preterm birth (Ruiz et al., 2003).

In our study, heifers had higher plasma bactericide activity, total plasma protein, IgM concentration, and acute-phase haptoglobin than their calves (Table 3.2; $P < 0.001$). Haptoglobin, immunoglobulins, and pro-inflammatory leukocytes are expected to change in parturient cow due parturition inflammation. However, heifers' plasma had greater bactericide activity compared to calves (Table 3.2; $P < 0.001$) due to their humoral immunity. Since humoral

immune response happens when B cells are activated and differ into antibody secretion cells after the contact with pathogens (Janeway et al., 2001). Calves will have less humoral immunity response than cows because their immune memory will just start to be developed after birth. In addition, a correlation was found between calf haptoglobin and plasma bactericide activity (Table 3.3; $r = -0.03$, $P < 0.05$), ratifying that the effectiveness of humoral immunity can be influenced by acute-phase proteins (Moisá et al., 2018). Immunoglobulins G1 concentrations were higher in calves than in heifers (Table 3.2; $P < 0.001$), probably because immunoglobulins were already transported to colostrum by time the heifers' blood were collected (Besser and Gay, 2014). However, a positive correlation was found between heifer IgG1 and calf cortisol (Table 3.3; $r = 0.29$, $P < 0.05$), suggesting that heifers had less IgG1 transported to colostrum increasing cortisol concentration in their calves.

Conclusions

In conclusion, behavioral measurements provided a better interpretation of calf passive immunity transfer acquired from maternal colostrum compared to heifer's and calf's immune and hematologic measures. Observation of calf behaviors is a simple and inexpensive tool that can be used by producers to improve the health and welfare of neonatal calves. Assistance is recommended for calves that are taking longer than 1 h to stand after birth. Hematological and immunological measures for cows and calves can be used as health indicators. Further studies on time spent suckling and amount of colostrum ingested may help to answer remaining questions about adequate passive immune transfer in beef calves.

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Tables

Table 3.1. Differences between calf behaviors and immune status, and cow immune status for calves that fail or pass passive immune transfer.

	Passive Transfer[1]			
	FAILED	**SUCCEED**	**SEM[2]**	***P* - values[3]**
Number of calves	**10**	**49**	**—**	**—**
Calf behaviors				
Latency[4] to:				
Stand, min	119.4	50.6	15.67	<0.001
First suckle, min	182.3	104.9	31.59	0.029
Last teat suckled, min[5]	222.3	135.6	39.59	0.014
Udder usage, %	86.1	90.8	6.57	0.52
Calf immune status				
Total Plasma Protein, %	6.4	10.2	0.41	<0.001
IgG1, mg/mL[5]	14.5	27.4	7.60	0.052
IgM, mg/mL[6]	0.6	1.8	0.58	0.052
Haptoglobin, ug/mL[5]	59.1	54.8	9.04	0.904
Cortisol, ng/mL[5]	18.1	14.9	5.48	0.479
Plasma Bactericide, %[6]	22.1	18.8	3.31	0.331
Cow immune status				
Total Plasma Protein, %	13.2	13.3	0.45	0.86
IgG1, mg/mL[6]	6.3	10.2	3.65	0.091
IgM, mg/mL	4.3	4.1	0.60	0.792
Haptoglobin, ug/mL[5]	59.1	54.8	9.03	0.852
Cortisol, ng/mL	14.5	16.7	2.83	0.555
Plasma Bactericide, %	31.3	30	3.72	0.746

[1]Passive immune transfer was calculated using IgG from BRIX (Morril et al., 2013) calves were separated in two groups using the threshold of 10 g/L of IgG (Failed < 10 g/L; Succeed > 10 g/L); [2]Largest SEM; [3]LS-means differ (Tukey-Kramer adjustment); [4]Time interval between birth and next behavior; [5]*P*-values derived from log-transformed values; [6]*P*-values derived from square-root-transformed values.

Table 3.2. Hematological and immunological parameters of Angus-cross heifers and calves.

	Heifer			Calf			
Parameters	**n[5]**	**Mean**	**SE**	**n[5]**	**Mean**	**SE**	***P*-value**
Hematocrit %	58	42.24	0.49	59	37.73	0.82	<0.001
TEC, x 10^6/uL[1]	58	7.84	0.10	59	8.29	0.14	0.01
Hemoglobin, g/dL	58	13.03	0.14	59	10.96	0.20	<0.001
MCV, fL[2]	58	53.62	0.43	59	45.30	0.36	<0.001
MCH, pg[3]	58	16.66	0.94	59	13.20	0.60	<0.001
MCHC, g/dL[4]	58	31.09	0.11	59	29.17	0.14	<0.001
Reticulocyte, x 10^3/uL	58	2.80	0.15	59	4.55	0.57	<0.001
Leukocytes, x 10^3/uL	58	11.04	0.37	59	9.96	0.42	0.059
Neutrophil, x 10^3/uL	58	5.39	0.35	59	6.52	0.33	0.024
Lymphocyte, x 10^3/uL	58	4.47	0.13	59	3.10	0.12	<0.001
Monocyte, x 10^3/uL	58	1.04	0.03	59	0.18	0.03	<0.001
Eosinophil, x 10^3/uL	58	0.13	0.01	59	0.02	0.16	0.745
Basophil, x 10^3/uL	58	0.003	<0.01	59	0.01	<0.01	<0.001
Platelets, x 10^3/uL	58	350.0	14.27	59	404.90	12.47	0.004
Neutrophil:Lymphocyte	58	1.29	0.10	59	2.16	0.10	<0.001
Cortisol, ng/mL	58	12.98	1.17	59	14.13	1.83	0.600
Haptoglobin, ug/mL	58	158.20	26.31	59	55.56	3.69	<0.001
Plasma bactericide %	58	30.17	1.53	59	19.36	1.38	<0.001
Total plasma protein, bryx	58	13.22	0.26	59	9.51	1.92	<0.001
IgG1, mg/mL	58	9.51	1.54	59	25.20	3.16	<0.001
IgM, mg/mL	58	4.17	0.25	59	1.58	0.24	<0.001

[1]Total erythrocyte count; [2]Mean corpuscular volume; [3]Mean corpuscular hemoglobin; [4]Mean corpuscular hemoglobin concentration; [5]Number of heifers or calves used in the analyses.

Table 3.3. Correlation statistics of calf behaviors, immune status, and cow immune status and gestation length.

	Calf immune status											
	TPP		IgG1		IgM		Hp		Cortisol		PB	
	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
Calf behaviors, latency[1] to:												
Stand, min	-0.45	<0.01	-0.16	0.24	-0.09	0.52	-0.03	0.8	0.14	0.3	0.15	0.27
First suckle, min	-0.24	0.08	-0.21	0.12	-0.2	0.15	-0.09	0.52	0.06	0.64	0.13	0.33
Last teat suckled	-0.17	0.22	-0.1	0.46	-0.11	0.44	-0.08	0.56	0.11	0.4	0.16	0.25
Udder usage, %	0.08	0.55	0.14	0.3	0.17	0.22	0.19	0.18	0.08	0.57	-0.03	0.83
Calf immune status												
Total plasma protein, % (TPP)	—	—	0.52	<0.01	0.52	<0.01	0.02	0.86	-0.21	0.12	-0.15	0.26
IgG1, mg/mL	0.52	<0.01	—	—	0.89	<0.01	0.16	0.21	-0.19	0.15	-0.19	0.16
IgM, mg/mL	0.52	<0.01	0.89	<0.01	—	—	0.09	0.5	-0.16	0.22	-0.21	0.11
Haptoglobin, ug/mL (Hp)	0.02	0.85	0.16	0.21	0.08	0.5	—	—	0.16	0.22	-0.30	0.02
Cortisol, ng/mL	-0.2	0.11	-0.18	0.15	-0.16	0.22	0.16	0.22	—	—	-0.12	0.37
Plasma bactericide, % (PB)	-0.15	0.26	-0.18	0.15	-0.05	0.7	-0.3	0.02	-0.12	0.37	—	—
Cow immune status												
Total plasma protein, % (TPP)	-0.03	0.81	-0.1	0.44	-0.07	0.6	- 0.03	0.84	-0.08	0.57	-0.14	0.3
IgG1, mg/mL	0.07	0.58	-0.07	0.58	-0.05	0.67	0.21	0.12	0.29	0.03	-0.2	0.15
IgM, mg/mL	-0.03	0.79	0.02	0.83	0.16	0.21	0.17	0.21	0.14	0.28	-0.17	0.21
Haptoglobin, ug/mL (Hp)	0.16	0.22	-0.01	0.93	0.06	0.66	0.03	0.85	-0.12	0.39	-0.23	0.09
Cortisol, ng/mL	0.05	0.7	-0.08	0.52	0.01	0.92	-0.1	0.47	-0.15	0.26	0.24	0.07
Plasma bactericide, % (PB)	-0.18	0.17	-0.18	0.15	-0.17	0.2	-0.03	0.82	0.15	0.25	-0.05	0.72
Cow												
Gestation length	0.11	0.37	0.07	0.59	-0.02	0.85	-0.11	0.43	0.24	0.07	0.33	0.01

First column correlates calf behaviors, calf immune status, cow immune status, and gestation length with calf measures of immunity; r = correlation coefficient; [1] Time interval from birth to next behavior.

Figures

Figure 3.1. Daily (mean ± SD) environmental temperatures and wind speed (top and bottom panels respectively) near Pollock, SD from March 18th to April 4th, 2018. Precipitation is represented with asterisks (snow) and solid circle (freezing fog) symbols.

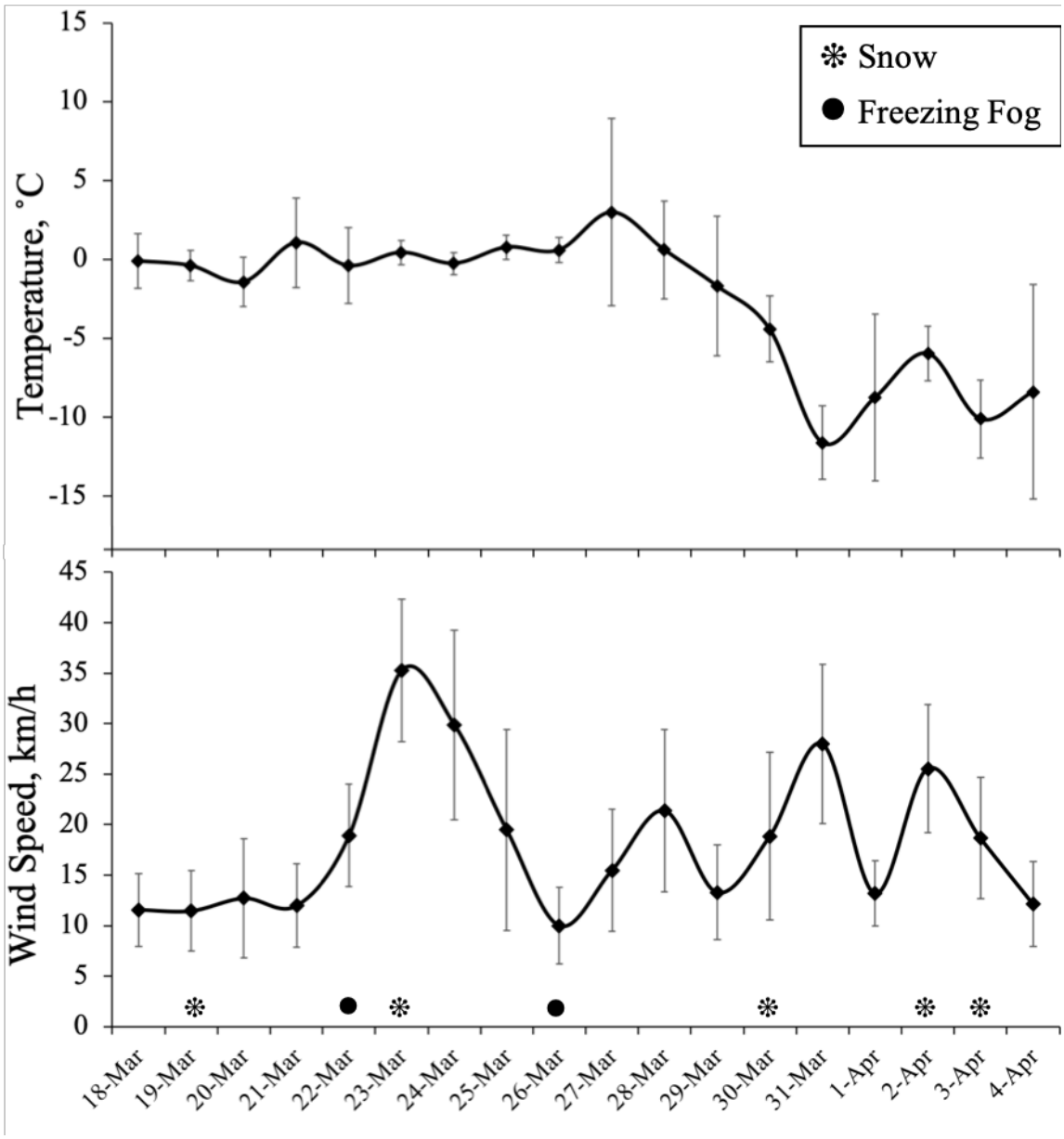
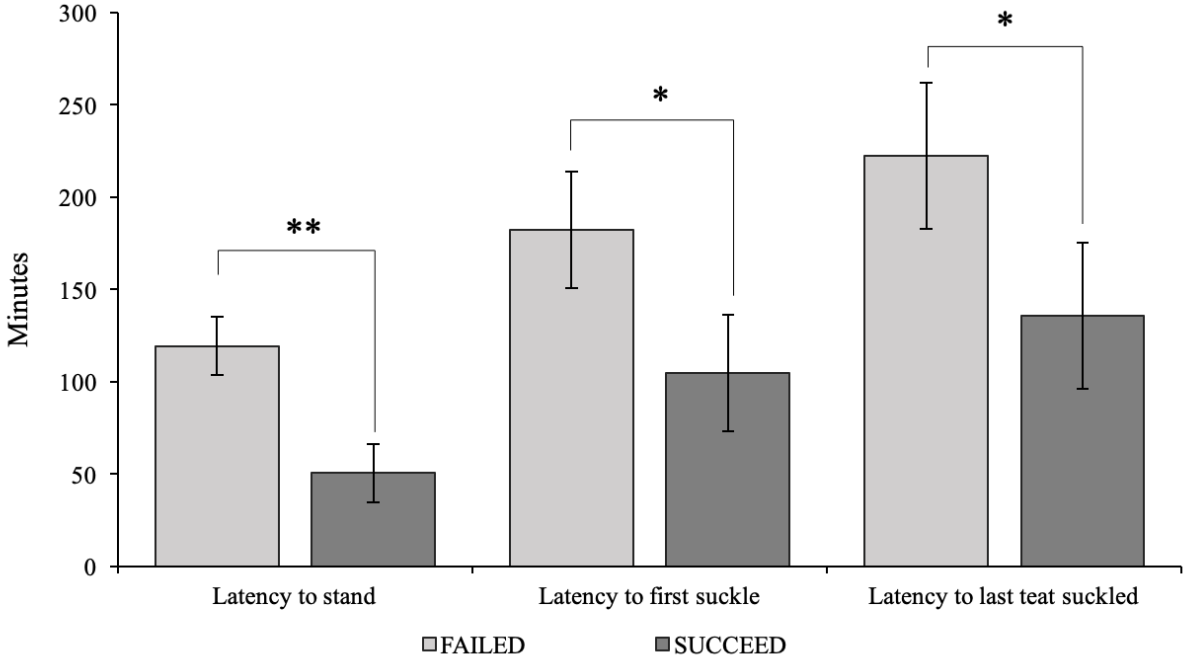


Figure 3.2 Neonatal behavior differences between calves that failed or succeed in receiving passive immune transfer from maternal colostrum.



Failure of passive transfer was defined as IgG < 10 g/L. Behaviors are represent by latency: time interval between birth and next behavior (stand; first suckle; and last teat suckled). LS-means differ (** $P < 0.001$; * $P < 0.05$; Tukey-Kramer adjustment). Latency to last teat suckled *P*-value derived from log transformed values.