Pharmacological and Behavioral Weaning Strategies to Reduce Stress in Beef Calves

Thesis

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Abstract

Weaning is considered the most stressful period in a beef calf's life, as well as the most difficult for a producer to manage. The most common weaning strategy in the American beef industry is abrupt removal of calves from their dams. This abrupt separation results in decreased calf growth and efficiency, and often morbidity manifested as bovine respiratory disease. Ultimately, this results in compromised calf welfare and increased production costs for beef producers. To evaluate alternative weaning strategies, both a pharmacological and a behavioral method to reduce stress were investigated.

In the first experiment, 80 heifer calves were randomly allocated to one of two treatments: Social Facilitator (SF): calves placed on pasture at weaning with a social facilitator or Control (CON): calves placed on pasture at weaning without a social facilitator. With four replications total, the social facilitator in each replication was a confirmed bred heifer. All calves were tracked using a Garmin® Astro 430/T5 GPS/GLONASS Dog Tracking System. Body weight, chute score, and exit velocity were recorded for two weeks post-weaning, and blood samples were taken to quantify non-esterified fatty acid and β-hydroxybutyrate concentrations. Calf behavior was also recorded using instantaneous scan sampling for two weeks post-weaning. Calves placed with a social facilitator at weaning walked fewer kilometers and devoted less time to

walking on day 0. Body weight, average daily gain, chute score, exit velocity, and β-hydroxybutyrate concentrations were not affected by the weaning treatment. Non-esterified fatty acid concentrations were, however, greater in the SF treatment group on day 7 and 14 post-weaning. The results from this experiment indicate that the addition of a social facilitator at weaning does decrease the distance and amount of time calves spend walking. Therefore, this weaning method may be beneficial to behavioral responses at weaning and calf production.

The second experiment evaluated the use of intranasal oxytocin, at weaning. Twenty heifer calves were randomly allocated to one of two treatment groups: Intranasal Oxytocin (OXT) or Intranasal Saline (SAL) administered at weaning. Once calves received their intranasal treatment on the day of weaning, they were all housed together on pasture for the duration of the experiment. All calves were again fitted with their own Garmin® Astro 430/T5 GPS/GLONASS Dog Tracking System. Body weight, chute score, exit velocity, and behavior was also recorded for two weeks post-weaning. Blood samples were taken and used to quantify non-esterified fatty acid, β-hydroxybutyrate, and cortisol concentrations. There were no differences among the treatment groups in regard to GPS data, body weight, average daily gain, chute score, exit velocity, β-hydroxybutyrate, or cortisol concentrations, however, calves in the SAL treatment group had greater non-esterified fatty acid concentrations on day 1 post-weaning. Therefore, oxytocin may have had a short-term effect on plasma metabolites related to energy balance.

Dedication

To my mom and grandparents:

Sheryl A. Nickles and John and Roberta Arnold

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Chapter 1. Literature Review

Introduction

The most common weaning strategy in the American beef industry is the abrupt removal of calves from their dams at approximately 180-220 days of age (Rasby, 2007). Abrupt weaning is characterized by immediate cessation of milk supply to the calf and complete maternal separation (Enriquez et al., 2011). Calves are simultaneously subjected to unfamiliar environments, new diets, basic production practices (i.e. vaccinations, castration, de-horning, transportation), and co-mingling with new calves while also being denied social contact and care by the cow. When cattle undergo prolonged periods of stress during routine management processes, it poses negative economic consequences for the producer (Burdick et al., 2011). Subjecting cattle to stressful periods such as weaning also impacts how society perceives the beef industry and the welfare standards currently implemented. Modern consumers are becoming increasingly concerned with food animal agriculture and the production processes being utilized by producers (Olynk et al., 2010). Livestock treatment and on-farm animal welfare are viewed as a few of the most important consumer concerns (Frewer et al., 2005). Therefore, current weaning practices should be further investigated in an effort to discover a management strategy that both improves calf welfare and growth, while still proving to be beneficial to the producer.

Stress experienced due to weaning is one of the most critical factors that negatively effects the growth rate of young calves (Campistol, 2010). Not only is growth hindered during weaning, but stress behaviors such as walking and vocalizing are increased (Haley et al., 2005; Price et al., 2003). These abnormal behaviors during maternal separation ultimately result in body weight losses several days post-weaning (Weary et al., 2008). In addition, stressed calves become susceptible to pathogens and succumb to diseases typically of respiratory and gastrointestinal origin during weaning (Campistol, 2010). Alternative weaning strategies that aim to reduce body weight losses and anxiety behaviors (i.e. walking, vocalizing) in young calves must be explored as these are obvious detriments to calf growth and efficiency. Minimizing stressful behaviors will prove to be beneficial as calf welfare and health will also be improved. Thus, both pharmacological and behavioral alternative weaning strategies were evaluated in this thesis as methods to minimize body weight losses and abnormal stress behaviors in calves.

Development of the mother-young bond

The survival of a newborn calf depends greatly on the establishment of a strong social bond with its dam (Enriquez et al., 2011). A calf's recognition and attraction to its mother begins immediately after birth (Enriquez et al., 2011). This bond is initiated by the cow and is characterized by mothering behaviors such as licking, supplying milk, providing warmth and protection, and synchronizing of activities (Newberry and Swanson, 2008; Lidfors and Jensen, 1988). A dam's reproductive success is improved by investing in the survival of her newborn through the provision of care and nutrition (Weary and Fraser, 1995; Trivers, 1974; Godfray, 1995).

The cow-calf bond is more defined in beef cattle than dairy and is maintained and reinforced by the high frequency of nursing events as well as the social association the calf keeps with its mother while growing. This bond is sustained in both the cow and calf through the release of hormones and neurotransmitters (Weary et al., 2008). The secretion of reproductive hormones such as estradiol, prolactin, and oxytocin in the blood are of vital importance for the establishment of the mother-young bond and maternal behaviors in mammals (Poindron, 2005). Oxytocin mediates the activation of pathways that are responsible for maintenance and continuation of maternal care due to the fulfilling nature of social contact, lactation, and other responses from the offspring (Insel and Young, 2001; Insel, 2003).

The development of the cow-calf relationship can be divided into three stages. The initial stage consists of the first several months in which the cow seeks close contact with her calf. This is followed by the second stage in which the calf is responsible for initiating nursing and most social contact. The last stage is where the cow begins rejecting some of the nursing attempts made by the calf until nursing stops completely (Trivers, 1974). The cow-calf bond lasts for months, even after the last stage in which the cow begins to reject nursing attempts by the calf. To evaluate the cow-calf bond, eight-month-old heifer calves were separated from their dams for three weeks (Veissier and Le Neindre, 1989). The authors discovered that after reunion of heifers and dams, the social interactions were minimally affected, even though nursing behaviors had stopped.

Synchronized behaviors in cattle

Cattle are known to be influenced by behaviors of other members of the herd. The influences conspecifics have on herd members or offspring can lead to synchronization of behaviors that eventually form into established behaviors of a population (Nicol, 1995). The phenomena of individuals in a population having the ability to influence the behavior on other individuals is also known as allelomimetic, or collective behavior. Collective behavior is also when the behavior of one individual results in the exhibition of the same behavior by another individual (Nicol, 1995). Synchronized behavior is not only observed in mature herd mates, but also through damn-offspring interactions. In many species of ungulates alloparental care is established in which one or a few dams stay with the offspring while the rest of the dams forage (Räsänen and Kruuk, 2007). Other examples of allelomimetic behaviors in domestic species include feeding behavior of hens (Hughes, 1971; Keeling and Hurnik, 1993), pigs (Kilgour, 1978), and ponies (Sweeting et al., 1985), as well as the grazing and resting behaviors of cattle (Benham, 1982). While these behaviors have been observed, the mechanisms underlying behavioral synchrony are not fully understood (Estavez et al., 2007). Stoye et al. (2012) also provided evidence that the degree of synchrony among cattle also depended on the time of day. Cattle were observed to be most synchronized in the morning and evening and least synchronized in the middle of the day, indicating that time of day influences herd synchrony (Stoye et al., 2012).

Weaning stress

Mammals are classified as either "K-strategists" or "R-strategists". While "R-strategists" are species that emphasize high population growth rates and produce many offspring, "K-strategists" have relatively stable populations and produce less offspring. Species that are K-strategists show very strong maternal characteristics and have purposeful mother-young bonds (Newberry and Swanson, 2008). Cattle are considered "K-strategists" as they have fewer offspring, but also because the cows are heavily invested in the care and survival of their calf. When this strong bond is discontinued by separation (i.e. weaning), atypical stress related behaviors are immediately observed in both the cow and calf. When discussing the types of stressors endured by livestock at weaning, they are generally divided into three distinct categories consisting of psychological stress, physical stress, and physiological stress (Carroll and Forsberg, 2007). Comingling and re-grouping of calves during transport or weaning, exposure to new environments, restraint, or noise stress is classified as psychological stressor. Interference with animal's ability to recognize members in their social group can create social anxiety in newly weaned calves (Mendl, 1999). Additionally, Hickey et al. (2003) demonstrated plasma cortisol concentrations were increased in calves when a small group of cows and their calves were separated from the herd.

Physiological stressors are characterized as anything that causes deviation from homeostasis in the body such as not meeting nutrient requirements or disruptions in the endocrine system (i.e. during the stress response) (Carroll and Forsberg, 2007). During the stress response, metabolic changes occur in the animal's body to redirect nutrients and energy towards the tissues such as the central nervous system and immune system that require nutrients to help cope with the stressor. This redirection occurs in the form of increased lipolysis and gluconeogenesis (Saposky et al., 2000). Physiological changes due to stress can have adverse effects on animal behavior, growth, metabolic, immune, endocrine, and reproductive abilities (Price, 2013).

The last type of stressor is physical, which is correlated with anything causing harm to the animal such as injury, temperature stress, feed restriction/thirst, and disease (Carroll and Forsberg, 2007). A major source of physical stress for calves during the weaning period is transportation. It is a common practice to immediately wean and truck calves to deliver them to a sale yard or feedlot. Stress caused by transportation results from noise, vibration, social re-grouping, crowding, climatic factors, restraint, loading and unloading, and feed and water deprivation (Swanson and Morrow-Tesch, 2001). Generally, calves experience all three types of stress (psychological, physiological, and physical) during the weaning period, making this a critical time for producers to take advantage of management methods to reduce stress as much as possible.

Stress and temperament effects on production

Large economic losses in the beef industry occur every year due to morbidity in feeder cattle. Morbidity results in decreased performance, increased costs associated with treating and handling sick animals, and if severe, mortality. A compromised immune system and a decreased feed intake during weaning makes calves more susceptible to pathogens and infectious agents. Due to calves having weakened immune systems, producers face significant health issues during the weaning/receiving period commonly manifested as bovine respiratory disease (BRD). Bovine respiratory disease is caused by several different viral and bacterial agents. Viral agents often pre-dispose cattle to bacterial infections that are associated with BRD (Duff and Galyean, 2007). Health problems due to BRD early in a calf's life have been reported to later affect them in the finishing phase. Montgomery et al. (1984) observed that BRD decreased marbling scores and quality grades. Roeber et al. (2001) supported this by reporting lower hot carcass weights, marbling scores, and yield grades in cattle that were treated for BRD more than once versus cattle that were never treated.

Stress responsiveness has also been linked to cattle behavior and temperament (Burdick et al., 2011). Temperament has been defined by Fordyce et al. (1988) as the reactivity of cattle to humans and novel environments. If cattle exhibit negative behavioral responses to humans or an unfamiliar environment, growth and productivity can also be negatively affected (Burdick et al., 2011). Fully understanding the relationship between stress and temperament can benefit the industry through improved cattle selection and development of management strategies to avoid the negative impacts temperament has on cattle growth and immune function (Burdick et al., 2011).

Temperament has been consistently evaluated with the use of chute scores, pen scores, and exit velocities (Burdick et al., 2011). The chute score method uses a number system to analyze temperament while cattle are in a squeeze chute with their heads held in the head gate (Grandin, 1993). The scoring system ranks cattle from 1-5 based on the amount of movement in the chute. A score of 1 is given if there is virtually no movement by the animal, and a score of 5 is given if the animal is continuously and violently struggling in the chute. Additionally, exit velocities define the rate that the animal leaves the chute system and travels a specified distance in m/s (Curley Jr. et al., 2006; Prayaga and Henshall, 2005). Depending on the protocol used, this distance can range from 1-2 meters (Burrow, 1997; Curley Jr. et al., 2006; King et al., 2006; Café et al., 2010; Café et al., 2011).

Several studies have attempted to relate chute scores and exit velocities to temperament and growth. Burrow (1991) reported that cattle with exit velocities faster than 2.4 m/s were considered temperamental, while cattle with exit velocities slower than or equal to 1.9 m/s were considered calm. Additionally, cattle with slower exit velocities have greater ADG than those cattle with fast exit velocities (Voisinet et al., 1997b; Burrow and Dillon, 1997; Müller and von Keyserlingk, 2006; Café et al., 2010). More excitable, temperamental cattle have lower live body weights (Fordyce et al., 1985), reduced feed efficiency (Petherick et al., 2002), poor meat quality (Voisinet et al., 1997a; King et al., 2006; Vann, 2011), and impaired immune function (Fell et al., 1999). Alternatively, cattle with calmer temperaments have been observed to have greater ADG (Burrow, 1997; Voisinet et al., 1997b; Petherick et al., 2002), greater female conception rates (Plasse et al., 1970; Cooke et al., 2011); and decreased dark cutting (Voisinet et al., 1997a; Scanga et al., 1998).

Typical maintenance behaviors of cattle

Several research teams have estimated the average total distances mature cattle walk on a daily basis (Squires, 1981; Hodder and Low, 1978; Ganskopp, 2001; Low et al., 1981b). Walking is considered a maintenance behavior, along with eating, ruminating, defaecating, urinating, drinking, and resting. Some of these behaviors such as grazing, occur in diurnal patterns which make them more predictable than others (Squires, 1981). In typical pasture-based systems, distance walked depends on forage quality, forage availability, and the distance from a water source. In extreme climatic situations of arid areas of Australia, stock have been recorded to travel up to 20 km/d to reach forage (Squires, 1981). In moderate to extreme climates on rangelands, cattle will typically walk about 7-14 km/d (Hodder and Low, 1978). This distance is generally lower, equal to 4-8 km/d in environments with more temperate climates and abundant forage (Squires, 1981).

Ganskopp (2001) estimated how far cattle would travel from resources by moving salt and water to different locations. The author found that separating salt and water versus keeping salt and water together did not affect the distance travelled by cattle each day and reported this daily distance to be 5.78 km. Ganskopp (2001) also reported that cattle typically remained no more than 2.13 km away from water and 2.58 km away from salt. Similar figures were reported with the majority of cattle grazing within 4 km of the water source, except for when forage supply was scarce and they travelled about 7 km (Hodder and Low, 1978). The authors also found that the greater distances recorded in their study were associated with either non-breeding cattle, or extreme drought. Additionally, Low et al. (1981b) reported that when the distance cows walked while performing no other activity was added to the distance walked while grazing, cows walked 9.3 km on average during a 24 hour observation period.

Certain behaviors are also more likely to be expressed during the day versus during the night with cattle. Grazing behavior of cows follow a basic pattern in which they graze heavily in the morning and evening, with minor additional grazing around noon and midnight (Favre, 1977). Longer day time grazing occurs in the early part of the day and late in the afternoon, as temperatures and radiation levels are typically lower (Low et al., 1981c). When forage was abundant, Low et al. (1981b) found that there tended to be more grazing and ruminating during the day and less resting. The authors (Low et al., 1981a) also reported that the timing of grazing bouts showed some seasonal differences, with typical grazing at dawn, dusk, and midnight supplemented with an additional grazing period at noon during the non-summer months.

Abnormal weaning behaviors and energy expenditure

While energy demands are extremely high during weaning, this is also a period where there is a clear depression in DMI. During abrupt separation (i.e. weaning), cows and calves both exhibit a shift in behavior that results in decreased time spent eating and resting, and increased time spent walking and vocalizing (Veissier and Le Neindre, 1989). Vocalizing and walking due to offspring being deprived of social interaction with their dams has also been consistently observed in other mammalian species (horses: McCall et al., 1985; Heleski et al., 2002; lambs: Orgeur et al., 1998, Orgeur et al., 1999; pigs: Weary and Fraser, 1997). Of the observable behavioral changes, vocalization and walking are considered the most prevalent during the weaning period. Vocalizations that occur immediately by calves after being separated from their dams are considered reliable indicators of the emotional and physical state of the calf due to the energy cost of vocalizing (Enriquez et al., 2011). High frequency vocalizations have also been associated with abrupt weaning strategies and represent calf frustration due to not being able to receive nutrition or care from the cow (Enriquez et al., 2010; Latham and Mason, 2008).

Pedometers were placed on calves to determine the amount of steps calves take during the day following weaning (Haley et al., 2005). The authors found that when they applied an average stride length of 65 cm, calves abruptly weaned walked approximately 16.7 ± 3.1 km/d on the first day after separation. When evaluating time devoted to walking, Price et al. (2003) reported significant effects for the time calves totally separated from their dams on pasture spent walking when compared to the other treatment groups (non-weaned controls on pasture, fence-line separation from dams on pasture, total separation from dams in dry lot preconditioned to alfalfa hay, and total separation from dams in dry lot but not preconditioned to hay). The authors discovered walking was typically around the groups' pasture or enclosure, and that the totally separated calves on pasture were observed walking more frequently than the other four treatments. Walking was discovered to be greatest on days 1 and 2, while gradually decreasing over days 3, 4, and 5 (Price et al., 2003).

Energy expenditure for maintenance has been previously defined as the energy required for basal metabolic processes plus some minimal amount of work, or as the amount of energy required to maintain energy balance without any amount of product formation (Ferrell, 1988; Baldwin, 1995). Once energy is consumed for maintenance processes, the extra energy is put into metabolic pathways that result in product formation (i.e. muscle, adipose, bone) (Caton et al., 2000). Growing animals allocate greater energy expenditure on protein synthesis for building muscle, resulting in greater total energy costs when compared to mature animals (Millward et al., 1976; Davis et al., 1981). The identification of energy consuming tissues and processes associated with maintenance and production should be helpful in creating management practices that aim to conserve energy expenditure. The conservation of this energy will result in more available energy for product formation (i.e. muscle), and less energy directed towards maintenance (Baldwin et al., 1980).

Several authors have estimated average maintenance costs in grazing ruminants (Osuji, 1974; di Marco et al., 1996). Ruminants on pasture spend more time eating and foraging for food than animals in confinement due to extra muscular activity. Depending on forage availability, it has been estimated that animals spend 7-10 hours a day grazing (Herbel and Nelson, 1966; Arnold and Dudzinski, 1978; Aello and Gómez, 1984; Lathrop et al., 1988) and spend 1-3 hours/day walking (Ribeiro et al., 1977). Increased activity time for grazing animals has been associated with a 25-50% increase in maintenance energy requirements (Osuji, 1974). The effect of grazing and increased activity will vary depending on how far the animal travels to find forage and how much of their time they spend walking and grazing (di Marco et al., 1996). In grazing sheep, Osuji (1974) reported that standing, walking, eating, and ruminating consumed 26.3% of total energy while confined sheep only spent 6.1% of total energy on these tasks.

Weaning methods

Beef producers typically wean their calves at approximately 5-8 months of age with the main objective of improving body condition scores of cows that are carrying a new fetus for the following calving season (Enriquez et al., 2011). Several factors such as pasture availability, forage quality, cow body condition scores, market prices, and family tradition influence a producer's decision about when to wean their calves.

The predominant weaning strategy used by producers is abrupt and permanent separation of the cow and calf (Lynch et al., 2010).

Several alternative weaning strategies are currently being proposed and researched as possibilities to minimize the stresses associated with traditional abrupt weaning, however, the results are equivocal. Fence-line weaning involves the denial of nursing, but still allows for social contact between cows and calves through a fence-line. Two-stage weaning utilizes anti-suckling nose-flaps that are placed in the nostrils of a calf and prevent nursing, while still allowing social contact with the cow. Price et al. (2003) reported that allowing fence-line contact between calves and cows on pasture for the first seven days of weaning reduced distress behaviors when compared with calves that were totally separated from their dams on pasture. The authors also reported that fence-line weaned calves had minimal weight losses in the days following separation from their dam. Opposingly, Enríquez et al. (2010), reported that fence-line weaned calves vocalized more than control and two-stage weaned calves. Two-stage weaning with nose flaps initiated two stress responses with the first being when calves were denied the ability to nurse, and second when calves were denied social contact with the dam (Enríquez et al., 2010). These authors also stated that control calves had greater ADG than fence-line and two-stage weaned calves, and thus concluded that neither of the alternative weaning methods provided benefits in reducing weaning stress.

Several other authors also evaluated the two-stage weaning method in which calves are first denied the ability to nurse with the use of a nose flap before complete separation from the dam. Haley et al. (2005) found that while two-stage weaned calves vocalized and walked less, and spent more time eating and resting after separation, there were no differences in overall ADG versus the control calves that were abruptly separated. Alternatively, Campistol et al (2013) stated that two-stage weaning when implemented with total separation from the dam could improve calf growth performance. Thus, while alternative weaning strategies are being proposed, the conflicting results regarding their efficacy must be taken into consideration.

The stress response

The hypothalamic-pituitary-adrenal (HPA) axis is responsible for initiating a response when calves undergo any type of stress. Hypothalamic-pituitary-adrenal axis activation results from internal and external stressors and causes an increased synthesis and release of corticotropin-releasing hormone (CRH) (O'Connor et al., 2000). This hypothalamic CRH is the peptide that is responsible for endocrine, autonomic, immunological, and behavioral responses to stress in mammals (Moberg and Mench, 2000). Anxiogenic effects have been observed in humans when there is hypersecretion of this CRH (O'Connor et al., 2000). Gupta et al. (2004) observed a stimulatory effect of bovine CRH first on the pituitary gland, and then the adrenal glands when CRH was administered to steers.

Oxytocin

In response to certain stressful stimuli, oxytocin has been observed to be released from the hypothalamo-neurohypophyseal system (Carter and Lightman, 1987; Gibbs, 1984; Higuchi et al., 1988; Sanders et al., 1990). Oxytocin is also found throughout the central nervous system and plays crucial roles in sleep/wake patterns, lactation, and sexual/maternal behaviors (Richard Ph Moos and Freund-Mercier, 1991). Due to oxytocin's widespread role in the body, there are several pathways throughout the brain regions that are associated with the stress response. This includes the bed nucleus of the stria terminalis, the amygdala, and the brain stem nuclei (Ingram and Moos, 1992; Condès-Lara et al., 1994; Siaud et al., 1989).

The majority of oxytocin in the body is stored in the posterior pituitary gland. This is the gland in which the nerve endings of the magnocellular neurons lie. Magnocellular neurons synthesize oxytocin and vasopressin with their cell bodies lying outside of the blood-brain barrier which makes these peptides ready to enter the blood (Leng and Ludwig, 2016). Oxytocin is stable in the plasma, and the body works to clear the peptide via the kidneys and liver (Leng and Ludwig, 2016).

The average half-life for oxytocin after natural release or those administered by single intravenous injections have been reported to range from 0.55-3.6 minutes in cattle (Wachs et al., 1984). Shorter half-life values may be attributed to oxytocin disappearing from the blood circulation by elimination from the central compartment through kidney and liver degradation, or from distribution into the periphery (Wachs et al., 1984). The half-life for oxytocin has also been recorded in various other species including rats and guinea pigs. In rats with intravenous doses of oxytocin up to 500 ng/kg, oxytocin was observed to disappear from the blood circulation with a half-life of 3-8 minutes (Morvin et al., 2008). However, the half-life of the peptide in the cerebrospinal fluid has been observed to be longer, lasting up to 28 minutes in guinea pigs (Jones and Robinson, 1982) and 19 minutes in rats (Mens et al., 1983).

While oxytocin has numerous roles in the sleep/wake patterns, lactation, and maternal behavior, it is possible that it may be responsible for regulation of the response to stressful stimuli (Windle et al., 1997). Oxytocin is released in response to many different stimuli. Oxytocin is also able to stimulate its own release, creating a positive feed-back mechanism, which is unusual as nearly all hormones are regulated through negative feedback mechanisms (Moos et al., 1984). Russell et al. (2003) demonstrated voles pretreated with oxytocin had elevated concentrations of plasma oxytocin ninety-five minutes after the original injection, again suggesting that oxytocin has positive feed-back capabilities.

Oxytocin's effect on behavior

Previous studies in mice and rats have supported the hypothesis that oxytocin in high doses can lessen anxiety behaviors (Uvnäs-Moberg et al., 1994) while also elevating the nociceptive threshold in rats that are placed in stressful environments (Uvnäs-Moberg et al., 1992; Petersson et al., 1996). Amico et al. (2004) demonstrated that when the oxytocin gene in female mice was deleted, increased anxiety behaviors were apparent when compared to wild type mice. Windle et al. (1997) discovered the anxiolytic effect oxytocin had on rats that had been exposed to noise stress. The authors found that oxytocin decreased the corticosterone response in a dose dependent manner in rats exposed to noise stress. Additionally, the authors hypothesized that oxytocin could have had a direct effect on the production and release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus. Therefore, oxytocin could be acting to inhibit the release of CRH itself, thus causing a down-regulation of the HPA axis and inhibiting the anxiogenic effects of CRH in the body (Windle et al., 1997).

While little work has been performed in livestock, the reported effects of oxytocin on behavior and the stress response are equivocal. Infused oxytocin decreased the cortisol response in non-lactating ewes that were exposed to an audiovisual stressor, indicating oxytocin had an anxiolytic effect (Condès -Lara et al., 1994). Alternatively, when pigs were repeatedly treated with intranasal oxytocin at 1, 2, and 3 days of age they exhibited increased aggression and agonistic interactions such as head pushing behaviors (Rault et al., 2011).

Oxytocin's effect on social relationships

While oxytocin has been observed to have anxiolytic effects, it also has important effects on social relationships and bonding. A study using voles presented by Grippo et al. at the 2007 Society for Neuroscience meeting evaluated stress reactions in female prairie voles living in either isolation or with a female sibling. Grippo et al. (2007) found that after the voles lived in their respective treatments for four weeks, the female voles that lived in isolation had greater stress, behavioral anxiety, and depression. The study was then continued when the voles living in isolation were given either oxytocin or saline every day during the last two weeks of the four-week living period. The authors discovered that the isolated voles given the oxytocin treatment no longer showed signs of cardiac stress, depression, or anxiety (Grippo et al., 2007). This provides evidence that in highly social species, disruptions in oxytocin signaling due to partner separation or isolation can result in anxiety and depression disorders (Hurlemann and Scheele, 2016).

Proposed alternative weaning strategies

As discussed throughout this literature review, there are several behavioral and physiological mechanisms mammalian species have in response to stress. The first alternative weaning method evaluated in this thesis was the use of a social facilitator cow at weaning to alter calves' behavioral responses to weaning. Cattle are gregarious animals, thus a further source of stress for calves is the removal from the hierarchical society in which they were raised. In this cow-calf hierarchy, the brood cows are the dominant figures in which calves follow and model their behaviors. Therefore, it has been proposed that using mature animals as "trainer animals" or "social facilitators" will have effects on performance, health, and behavior of feedlot calves (Loerch and Fluharty, 2000). The reported results on the efficacy of trainer cows is equivocal. Loerch and Fluharty (2000) completed several trials evaluating the effect of both trainer cows and steers on calf performance, health, and behavior. It was reported that the use of feedlot adapted trainer steers was not beneficial. Alternatively, the authors did discover that in some of the trials, trainer cows had positive short-term effects on ADG, morbidity, and eating behaviors of calves. While these effects were short-term, the authors hypothesized that the effects may still have been sufficient in moderating stress and making the transition into the feedlot less difficult for calves. Schwartzkopf-Genswein et al. (1997), however, reported that while trainer cows did alter calf eating behavior, they did not observe an effect on performance or health status of calves. Additionally, Gibb et al. (2000) found negative consequences of using trainer cows as they observed calves penned with mature cows lying less frequently and appearing to avoid the cow at the feed bunk. The authors' results indicated that having a foreign mature cow can even be detrimental in settling calves into an unfamiliar environment. Thus, the mixed results examining the use of trainer cows and the lack of work done in pasture-based systems suggests that further evaluation is necessary.

The second proposed weaning strategy in this thesis involves the use of intranasal oxytocin to alter calves' physiological response to stress. It has been suggested from previous studies in humans and lab animals that the administration of endogenous oxytocin can regulate the stress response and maintain homeostasis in the body. While literature is limited in livestock animals, there have been a few studies evaluating oxytocin's ability to modulate the stress response, thus improving animal welfare. For example, Sutherland and Tops (2014) evaluated the possible involvement of oxytocin in modulating the stress response in lactating dairy cows. The authors discovered that when cows were exposed to a novel environment (new milking parlor), oxytocin concentrations were initially decreased and then increased over days of milking in the novel environment. This increase in oxytocin was also associated with a decrease in cortisol. This study suggests that oxytocin plays a role in habituation to psychological stressors such as novel environments and experiences. Thus, if a cow has a greater oxytocin response to a stressor she may be better equipped to habituate to the stressor (Sutherland and Tops, 2014). Therefore, these along with other preliminary findings support the hypothesis that oxytocin administration or increased oxytocin release may be beneficial to livestock as it can help attenuate the stress response and allow for positive social interactions and behavior.

Utilizing GPS Technologies

Satellite-based global positioning systems (GPS) have been used since the late 1980's to provide unique data providing opportunities to track animal movement (Swain et al., 2011). Global positioning systems allow researchers to track animals as well as their interactions with the environment. A global positioning system works by receiving signals from orbiting satellites and using distance measures from these satellites, the GPS device can then calculate the location in relation to the satellites (Swain et al., 2011). These systems have been used by a wide array of researchers in the evaluation of both wildlife and livestock. Wildlife ecologists have previously used GPS devices to track animals in extensive landscapes, while animal scientists have used GPS devices to provide data about livestock behavior, mainly in grazing experiments (Swain et al., 2011). Using GPS devices to track livestock is easily controlled by the researcher, as they can ideally control the animals and replicate treatments (Swain et al., 2011).

To track an animal's movement, a GPS receiver must be fitted and programmed to record a GPS location in fixed intervals. These data points from each individual interval is then used to construct the movement pattern completed by the animal (Swain et al., 2011). The accuracy of this pattern is related to the frequency of the fixed intervals in which data points are taken. The more frequently data points are taken, the more accurate the reconstruction of the animal's path will be (Swain et al., 2011). Shorter fix intervals are associated with higher fix success rates and better GPS accuracy (Moen et al., 2001). Several other factors impact GPS accuracy such as number and position of visible satellites and the proximity of the satellites (Pépin et al., 2004). By correlating the animal's behavior with environmental information, researchers can then determine optimal management strategies (Handcock et al., 2009).

In cattle, recent work has been performed using high sample-rate GPS and exploring the link between prediction error and GPS fix-rate (Swain et al., 2008). This study reported that a GPS location needs to be collected at least once every 10 seconds to obtain at least 90% accuracy. These technologies can be extremely helpful in connecting the gap between theoretical constructs and true observations that can then solve many applied agricultural and environmental problems (Guo et al., 2009). Additional work in cattle has been performed by Ganskopp (2001) that evaluated the effectiveness of using salt and water placement as manipulations for the distribution of cattle. This study utilized GPS collars to obtain cattle's movements and activities in proximity to water and salt. Using the GPS collars, Ganskopp (2001) was able to determine the mean distance of cattle from salt and water sources as well as distance travelled per day, and time spent resting and grazing per day. The authors concluded that cattle were more likely to make efforts to remain near the water source rather than the salt source. Additionally, Turner et al. (2000) monitored cattle behavior and pasture use with GPS collars. Turner et al.

(2000) hypothesized that using GPS collars and obtaining precision animal locations would help the authors evaluate grazing behaviors and pasture utilization to greater understand animal performance. Using the GPS collars, the authors were able to determine that the patterns displayed implied that cow movement was influenced by ambient temperature, and that there were definitive grazing preferences exhibited by cows on pasture. Furthermore, Ungar et al. (2005) utilized GPS technology to determine the distribution of time cattle spent on certain behaviors in both the United States and Israel. The authors reported that in both countries, standing and lying accounted for almost half of the total observations, grazing accounted for 45% in the United States and 41% in Israel. Ungar et al. (2005) also found that travelling accounted for 6% and 10%, respectively, and drinking and mineral consumption in both countries accounted for less than 1% of total observation time. Thus, GPS is a promising, innovative technology to help characterize the behavioral and walking patterns of cattle.

Conclusion

Weaning is physiologically and behaviorally stressful on both cows and calves, however, it is a customary practice in the beef industry to improve cow body weight and reproductive success. More research on alternative weaning methods that are viable options for producers to reduce stress while also still being profitable is necessary. If ignored, decreased efficiencies and increased morbidity during weaning will result in greater labor and health costs for producers, as well as increased days on feed for calves to reach harvest weights. Examining this period further and identifying alternative weaning strategies such as the use of oxytocin or social facilitator cows could result in less economic loss for producers and may develop more consumer trust in the beef industry's animal care standards. Therefore, intranasal oxytocin and the addition of a familiar social facilitator cow during weaning may have the ability to modify calves' behavioral and physiological responses, ultimately making this period more profitable for producers and less stressful on calves.

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Chapter 2. Abruptly weaned calves walk lesser distances and devote less time to walking in pastures when placed with a social facilitator cow

Abstract

Effects of a social facilitator cow on the body weight and behavior of weaned beef calves were evaluated in a completely randomized block design experiment. In the present study, there was allocation of 80 Simmental x Angus heifer calves in four replications. In each replication, ten abruptly weaned heifer calves were randomly assigned to either the social facilitator (**SF**) treatment in which a familiar, pregnant non-lactating cow was placed with the calves on pasture on the day of weaning (day 0), or ten abruptly weaned heifer calves were randomly assigned to the control (**CON**) treatment group without a SF cow being joined with the calves on pasture on the day of weaning (day 0). All calves were weighed and blood sampled on days 0, 1, 7, and 14. Blood samples were subsequently used to quantify non-esterified fatty acid (NEFA) and β-hydroxybutyrate concentrations. To evaluate calf behavior, chute scores and exit velocities were recorded on days 0, 7, and 14. To evaluate and quantify walking behaviors commonly associated with weaning, all calves were fitted with an individual global positioning system collar that recorded calf location every ten seconds for a 24 hour period on days 0, 7, and 14. Additionally, calf behavior was examined with instantaneous sampling on days 0, 7, and 14 from 0730 to 0830, 1200 to 1300, and 1700 to 1800 h. The inclusion of the social facilitator with the calves at weaning resulted in a decrease the distance calves walked on day 0 post-weaning ($P < 0.01$), as well as the time calves devoted to walking ($P = 0.03$). The SF treatment group also had greater NEFA concentrations on d7 and d14 post-weaning ($P = 0.01$), however, there were no differences observed for body weight or average daily weight gain. The results of this study indicate that the addition of a social facilitator animal at weaning alters the distance and total time calves devote to walking during the first day after weaning, but this change was not enough to affect calf growth.

Key Words: beef cattle, behavior, global positioning system, welfare

Introduction

Current production practices in the beef industry are centered on the weaning of calves between 5 and 8 months of age (Enríquez et al., 2011). The goal of weaning beef calves is to improve reproductive success and body weight of cows (Enríquez et al., 2011) while also preparing calves for the receiving, growing, and finishing phases of production. Cows and calves that have been abruptly separated express behaviors indicating there is a strong desire to maintain the social bond with each other, as beef cows express strong maternal characteristics as a result of behavioral responses developed throughout evolution of the species (Newberry and Swanson, 2008). Both cows and calves vocalize repeatedly in response to separation and are observed in devoting more time to walking, and less time to behaviors such as eating and resting during a period of several days subsequent to weaning (Veissier and le Neindre, 1989). The behavioral responses such as walking and vocalizing of both cows and calves are greatest during the first 1 to 2 days after separation but in some cases these behaviors continue to occur for several days after weaning (Weary et al., 2008). Abnormal behaviors in response to the stress of being separated from each other as a result of weaning ultimately result in body weight loss for several days post-weaning and a subsequent reduction in body weight growth (Weary et al., 2008). The behavioral and physiological responses to weaning can further result in impaired metabolic, immune, endocrine, and reproductive capacities (Price, 2013). These pronounced responses to weaning are an obvious impediment in maximizing calf production and efficiency, as well as in reducing stress that is related to calf and cow welfare that are concerns in the industry (Weary et al., 2008).

Cattle are affected by behaviors of other conspecifics of the herd. The phenomena of individuals in a population having the capacity to influence the behavior on other individuals is termed allelomimetic, or collective behavior. Cattle express these collective behaviors through allogrooming (Sato et al., 1993), alleloparenting (Sato et al., 1987; Castanheira et al., 2013; Finger et al., 2014), and allosuckling (Castanheira et al., 2013) of calves. The behavior of conspecifics can lead to the synchronization of the timing of behaviors with those of other herd conspecifics that eventually result in established behaviors for a population (Nicol, 1995). One strategy associated with utilizing the natural allelomimetic behavior in cattle is the use of a social facilitator or trainer animal during times of stress, although the reported results thus far have not been consistent. The addition of a trainer cow had positive short-term effects on ADG, morbidity, and eating behaviors of calves but the addition of a trainer steer had no effect (Loerch and Fluharty, 2000). Additionally, Gibb et al. (2000) reported that the addition of trainer cows in a feedlot setting with newly weaned beef calves did not result in improvement of calf health, time spent at the feed bunk, or calf body weight gain. The variability in these results could be due to differences in the management of the trainer cows at the start of the study, as Loerch and Fluharty (2000) used non-pregnant cows and followed a protocol to keep them from displaying estrus during the experiment, however, Gibb et al. (2000) used pregnant cows in two of the experiments and non-pregnant trainer cows in one experiment with no indication of estrus prevention in the non-pregnant cows. Trainer cows and trainer steers used by Loerch and Fluharty (2000) were also given a three week adaptation period to the feedlot prior to arrival of the newly weaned calves, while the cows used by Gibb et al. (2000) were only allowed a week for adaptation to the feedlot. Both of these previous experiments also used trainer cows (Loerch and Fluharty, 2000; Gibb et al., 2000) and trainer steers (Loerch and Fluharty, 2000) that were not familiar to the calves and had no prior contact with the weaned calves before the start of the experiment. Previous studies have demonstrated that cattle are efficient in individual recognition (Coulon et al., 2009), and can use the identities of individual familiar conspecifics as cues in a discrimination learning test (Hagen and Broom, 2003). It has also been reported that relative dominance ranks of individual steers in confinement could be determined after a few hours of observation, indicating that the social hierarchy in cattle is established quickly (Stricklin et al., 1980). Thus, familiarity of the social facilitator animal to weaned calves may be important because the grouping of unfamiliar animals causes aggression, social stress, and increased walking behaviors (Bøe and Færevik, 2003), and could add additional stress during the weaning period. Furthermore, the effects of a familiar social facilitator animal on weaned beef calves in a pasture-based production system is unknown. Thus, the objective of the present study was to determine the effects of a familiar social facilitator on calf behavior, body weight and plasma metabolites such as non-esterified fatty acids and β-hydroxybutyrate. The hypothesis was that placement of calves with a familiar social facilitator cow in pastures would induce allelomimicry behavior (i.e., behavior synchronization) between the social facilitator and the group of calves. It was also hypothesized that there would be a decrease in the distance and time calves devoted to walking after weaning with inclusion of a familiar social facilitator in the pasture, thus having a positive effect on calf body weight, average daily gain, and plasma metabolites such as non-esterified fatty acids and β-hydroxybutyrate.

Materials and Methods

All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee (Animal Use Protocol #2018A00000085). The following study was completed in 2018 at the Jackson Agricultural Research Station (JARS; Jackson, OH, USA) and the Eastern Agricultural Research Station (EARS; Caldwell, OH, USA). Two replications of each treatment were conducted at the JARS, and two replications were conducted at the EARS. All four replications between the EARS and JARS were completed within a 9 week period.

Animals and Management

A total of 80 Angus x Simmental heifer calves (182.3 ± 9.53 kg, 184 ± 25 days of age) were included in the study with 40 being at the JARS and EARS, respectively. Before calves were weaned, they were maintained with their dams on tall fescue pastures. All four replications were completed from late August to mid October 2018, therefore sufficient forage was available for calves to graze during all replications. During the study, calves remained at the research stations at which they were born and were used for the replications at the respective station. All calves were treated similarly before the weaning study was initiated and were vaccinated with a modified live virus (Bovi-Shield Gold 5, Zoetis Services LLC., Parsippany, NJ, USA) against IBR, BVD (Types 1 and 2),

PI_3, and BRSV viruses, and a bacterin-toxoid for prevention of bovine pneumonia (One Shot, Zoetis Services LLC., Parsippany, NJ, USA). All calves were also de-wormed at the time vaccination was conducted.

Heifer calves were randomly allocated to each treatment group (n = 10), social facilitator (SF) or control (CON) on the day of weaning (d 0) in each replication of the study. Calves in the SF group were placed on pasture at weaning with the familiar social facilitator, and control calves were placed on a similar area of pasture without a social facilitator animal placed with the calves of this group. Pasture dimensions at the JARS were similar among replications of the study, with calves in each treatment group being placed in a pasture that was 1 hectare in size. Pasture dimensions at the EARS were similar for both replications of the study with calves being placed in a pasture that was 0.4 hectare in size. At weaning, calves on trial at the JARS and EARS were provided 0.91 $kg \cdot animal^{-1} \cdot day^{-1}$ of a similar 17% protein starter diet (Table 1).

The social facilitator used in each replication were pregnant heifers that were also from the respective research herds. A confirmed pregnant heifer was used to avoid the possibility of the trainer animal expressing estrus while the experiment was being conducted. The social facilitator was placed in the pasture with the calves and their dams a minimum of 3 days before weaning was initiated to ensure there was familiarity of the social facilitator animal and the heifer calves.

Global positioning system collars

All calves in each group (SF, CON) and each social facilitator animal had a collar placed around their neck with a Garmin global positioning system (GPS) tracking device

(Garmin International, Inc., Olathe, KS) at weaning on day 0. Each collar remained on the calves for a 24-hour period and was then removed to perform battery recharging. The collars were on the calves and the social facilitator animal for 24 hours on days 0, 7, and 14 and were subsequently removed on days 1, 8, and 15. The GPS devices were calibrated to record the position of the calves every 10 seconds. The data extracted from the collars were total distance travelled (km), walking time (h, total time spent walking), non-walking time (h, total time spent on all other activities besides walking), area utilization index (AUI-calculated by determining the area that each individual calf was tracked on days 0, 7 and 14 and dividing this area by the total area of the pasture), and average speed (m/s). This data were obtained using the Garmin BaseCamp software (Garmin International, Inc., Olathe, KS).

The collars used were the Garmin Astro 430/T5 GPS/GLONASS Dog Tracking System. The collars were reported by Garmin to be accurate to within 15 meters 95% of the time when there were no physical obstructions of the system to the celestial sphere. The Garmin documentation about the device also indicated “Generally, users will see accuracy within 5-10 meters under normal conditions.”

Behavioral Observations

There was recording of behavioral observations using an ethogram (Table 2) on days 0, 7, and 14. On days 0, 7, and 14 there was recording of behavioral observations from 0730 to 0830, 1200 to 1300, and 1700 to 1800 h with an instantaneous sampling technique (Lehner, 1979) every 10 minutes throughout the duration of the hour (seven total scan samples/hour) to provide observations in the morning, afternoon, and evening.

For analysis, values for behaviors for an entire day of scan sampling were totaled and subsequently divided by the total number of counts that day to provide a relative count of how many calves were expressing specific behaviors during the observation periods for each treatment group. For example, if there were 10 counts recorded for calves feeding, the relative count for that observation day would be calculated as follows:

$$\frac{(10 \text{ recorded counts})}{\left(7 \frac{\text{scan samples}}{\text{h}} \times 3 \text{ h}\right)} = \frac{10}{21} = 0.48$$

Sampling and Analysis

The sampling timelines and procedures were identical for all four replications. All calves were blood sampled (10 mL) via jugular venipuncture and body weight was quantified on days 0, 1, 7, and 14. There was collection of blood samples in vacutainer tubes containing lithium heparin. The samples were then placed on ice and transferred back to the laboratory where the tubes were centrifuged for 25 minutes at approximately 1,800 *x g* and 4°C. The plasma was then further aliquoted into individual microcentrifuge tubes to determine non-esterified fatty acid (NEFA) and β-hydroxybutyrate (β-HB) concentrations. Plasma NEFA concentrations were determined using micro titer plates and a plate reader in a 2-reaction, enzyme-based assay (NEFA-HR(2), FujifilmWako Diagnostics, USA, Richmond, VA) as previously described by Johnson and Peters (2003). Plasma β-hydroxybutyrate concentration was quantified using LiquiColor procedure (Beta-Hydroxybutyrate LiquiColor Test Endpoint, Stanbio Laboratory, Boerne, TX). For NEFA, inter-assay variation was 5.6% and intra-assay variation was 4.8%. For β-hydroxybutyrate, inter-assay variation was 12.0%, and intra-assay variation was 8.4%.

Chute Score and Exit Velocity

To evaluate behavior, chute scores and exit velocities were recorded in real time by a single observer and with the use of an infrared sensor on days 0, 7, and 14. A method described by Grandin (1993) was used to visually determine chute scores. This system was based on a range of 1 to 5 while the calves were restrained in the handling system: 1 = calm, no movement; 2 = restless, shifting; 3 = squirming, occasional shaking of the chute; 4 = continuous vigorous movement and shaking of the chute; 5 = rearing, twisting of the body, or violent struggling. There was recording of exit velocities using a method previously published by Burrow et al. (1988) in which exit velocity was quantified using infrared sensors (FarmTek, Inc., Wylie, TX). The “start” sensor was placed approximately 0.6 meters from the head gate of the chute, and the “finish” sensor was placed approximately 1.83 meters from the starting sensor. The exit velocity was recorded as the amount of time it took each calf to travel the 1.83 meters and the exit velocity was expressed in seconds.

Statistical Analysis

Calf body weight, average daily gain, NEFA concentration, β-hydroxybutyrate concentration, chute scores, exit velocities, and calf collar data (distance, walking time, non-walking time, pasture utilization index, average speed) were all analyzed as a randomized complete block design with repeated measures using the proc MIXED procedure of SAS (9.4, SAS Inst. Inc., Cary, NC). The model included treatment, day, and their interaction as fixed effects, and block (site of replication), calf ID, and pen as random effects, and pen as experimental unit. The calf collar data model included the group statement in SAS to include day as a covariate. Calf behavior data were analyzed as a randomized complete block design with repeated measures (proc MIXED procedure, SAS 9.4, SAS Inst. Inc., Cary, NC). The model included treatment, day, and their interaction as fixed effects, and block as the random effect. Analyses for all models were conducted using different covariance structures and the model with the lowest AIC was selected. Differences were considered to have resulted from the treatments if $P \leq 0.05$. Tendencies were considered to have resulted from the treatments if $P \leq 0.1$ and > 0.05. Descriptive statistics for the facilitator animal's GPS collar and behavioral observation data are shown in Table 3.

Results

Global positioning system collar data

The data obtained from the calves' GPS collars with the treatment, day, and treatment x day interactions and the respective P values are presented in Table 4, while the descriptive statistics from the social facilitator animals are presented in Table 3. There was a significant treatment × day effect ($P < 0.01$) for distance walked, as calves placed with a familiar social facilitator walked less (7.19 km) on day 0 when compared with control calves (9.02 km). There was a treatment × day effect ($P < 0.01$) for walking time, with the calves that were placed with the social facilitator animal devoting less time to walking on day 0 when compared with the control calves (2.46 compared with 3.14 h). Time devoted to walking also decreased in both treatment groups from day 0 to day 7, and day 7 to day 14. There was also a significant treatment × day effect ($P = 0.05$) for non-walking time, with values for non-walking time being greater on day 0 for the social facilitator than the control group (21.71 compared with 21.00 h). There was a treatment × day ($P = 0.05$) effect for Area Utilization Index (AUI), with AUI decreasing from day 0 to day 7, and day 7 to day 14 in both treatment groups. The value for the AUI of calves in the SF group was less on day 14 than all other values. There were no treatment or treatment × day effects for average speed, however, there was a day ($P < 0.01$) effect with average speed in both treatment groups being less on day 0 and greatest on day 14.

Behavior Observations

The values for mean relative behavior counts from the 3 days of visual observations of calves (0, 7, and 14) and the respective P values are presented in Table 5, while the descriptive statistics from the social facilitators are presented in Table 3. There was a treatment × day ($P = 0.04$) effect for walking, as CON calves had more walking observations on day 0 compared with all other days. There was a day ($P < 0.01$) effect for vocalizing, such that calves in both treatment groups vocalized only on day 0 and there were no vocalizations on days 7 and 14. There were no treatment, day, or treatment × day effects for standing, lying, or drinking.

Chute Score and Exit Velocity

Chute score and exit velocity data are presented in Table 5. There were no treatment, day, or treatment × day effects on chute score between either treatment group. There were also no treatment or treatment × day effects on exit velocity, however, there was a day effect ($P < 0.01$) with exit velocity being different between the two treatment groups on day 8.

Plasma Metabolites and Growth Performance

The plasma metabolite, body weight and average daily gain data with the treatment, day, and treatment × day interactions and the respective P values are presented in Table 6. There was a treatment × day ($P = 0.01$) effect for plasma NEFA concentrations. The NEFA concentrations were similar on days 0 and 1 and were different on days 7 and 14, with concentrations being greater on these days in the SF calves. There was no treatment effect on β-hydroxybutyrate, however, there was a day ($P < 0.01$) and a treatment × day ($P = 0.04$) effect with day 7 values being greater in the SF group compared with the CON group (323.2 compared with 279.6 ± 23.48). There were no treatment or treatment × day effects on body weight, but there was a day effect ($P < 0.01$). There were also no treatment, day, or treatment × day effects for average daily gain.

Discussion

The present study supports the hypothesis that a familiar social facilitator animal placed with a group of calves at weaning, decreases the distance walked and amount of time devoted to walking by the calves. Personal observations and data from the present study corroborate the allelomimicry by the calves of the SF group because there was synchronization of calf activities with that of the SF cow. It is important to note that these are relative counts, and do not reflect the behavioral patterns of the entire day, rather just the three hours that the two treatment groups were observed on days 0, 7 and 14, however, the behaviors were synchronized between the SF calves and the SF cow. Behavior observations recorded during three 1-hour periods (morning, noon, and evening) were considered sufficient to capture relative behavior counts in the present experiment since daily grazing and activity cycles in cattle tend to be very consistent, with major grazing events occurring in the morning from 0400 h to 0800 h, and in the evening from 1800 h to 2100 h, and minor grazing events occurring from 0100 h to 0200 h and around 1200 h (Lyons and Machen, 2000). Allelomimetic behaviors have been recorded in domesticated species and include feeding behaviors in hens (Hughes, 1971; Keeling and Hurnik, 1993), pigs (Kilgour, 1978), and ponies (Sweeting et al., 1985), as well as the grazing and resting behaviors of cattle (Benham, 1982). The mechanism underlying the synchrony of behaviors in these species is not fully understood (Estavez et al., 2007). The GPS collar data in the present study, however, indicates a similar distance walked and a similar time devoted to walking for the calves of the SF group as the SF cow on days 0, 7 and 14. It is noteworthy that the SF cow decreased the distance she walked between day 0 and 7 indicative of her exploratory and or reestablishment behavior with her herd mates on day 0. This result causes us to further question the role of the social facilitators, and how this role may affect the equivocal outcomes from previous studies (Loerch and Fluharty, 2000; Gibb et al., 2000) In particular, for the results of the present study, the question was considered as to if the SF adult animal had been familiar with the weaning area on day 0 as was the case on day 7 and 14, would the effect on the calves placed with the SF animal have been greater than that in the present study? This is beyond the scope of data that were collected in the present study, nevertheless the calves of the SF group walked a lesser distance (7.19 km) compared with the control calves (9.02 km) on day 0. In typical pastoral settings, the daily distance walked by cattle is dependent on forage quality, forage availability and distance from a water source. In grazing systems with abundant forage and temperate climates, cattle will walk 4 to 8 km each day (Squires, 1981). In moderate and extreme climatic environments on rangelands cattle have been recorded to walk 7 to 14 km/d (Hodder and Low, 1978) and 20 km/d (Squires, 1981) to graze pastures. In the present study, there was no recording of the distance calves walked prior to weaning, however, on day 7 and day 14 post-weaning when calves were more acclimated to the weaning process, the distance walked was similar to that reported by Squires (1981) for cattle in temperate climates. Results from other studies indicate there were greater observational counts for walking on the day of abrupt weaning compared with non-weaned controls (Price et al. 2003) and this is consistent with the observational data of the present study. In addition, estimated walking distance was greater for abruptly weaned calves on the day of weaning compared to when there was a two-stage weaning process for calves (Haley et al. 2005). It is likely that a return to pre-weaned daily walking distance occurs within 3 days after abrupt weaning (Haley et al. 2005).

There are common exploratory behaviors when there is placement of animals in new environments. There has been further categorization of these behaviors as extrinsic or intrinsic exploration. Extrinsic is characterized by behaviors aimed at obtaining information about food availability or nesting sites, while intrinsic is considered exploration behaviors that result from the desire to investigate novel places or objects (Wood-Gush and Vestergaard 1989). Regardless of the motivation behind exploration of a new environment, it is hypothesized that an animal's familiarization with its environment provides information about resources (Barnett, 1963) and can possibly lead to the animal developing a cognitive map (Bell, 1991) that is important for survival being particularly the case during the evolution of the species. There has been documentation of exploratory behavior in pigs (Wood-Gush and Vestergaard, 1989), and there is the thought that cattle and sheep living in extensive agricultural conditions also exhibit these behaviors as a survival adaptation (Wood-Gush and Vestergaard, 1989). It is common for cattle released into a new area or pen to walk the perimeter fence (Price et al. 2003) and this was the observation on day 0 of the present study. The calves in both groups in the present study, however, utilized almost the entire area available on day 0 but there was a decrease in the area utilized with each subsequent sampling day. Furthermore, the calves of the SF groups had the least (0.56) area utilized on day 15. The present findings may be indicative of calves expressing exploratory behaviors on day 0. After calves are familiar with their environment and resources, it seems that they decrease their exploratory behaviors as evidenced by the decreased AUI on the subsequent sampling days.

The data in the present study do not support the original hypothesis that calves placed with a social facilitator animal would have decreased NEFA and ketone concentrations. Fasting or feed restriction will greatly increase free fatty acid concentrations in the blood (Newsholme and Start, 1973). The time required to return the NEFA concentration in the blood of a fasted or feed-restricted animal to the original state is delayed and may take as many as 8 days (DiMarco et al., 1981; Yambayamba et al., 1996). The duration of the NEFA realignment period, however, is dependent on the nutrient density of the diet during this period (DiMarco et al., 1981). The fescue-based diet of the calves in the present study was supplemented with a small amount of starter diet once daily (0.91 kg·animal^{-1}·day^{-1}; Table 1). The starter diet available for the calves in the present study was not as nutrient rich as the diets fed in previous studies where the effect of the NEFA realignment was assessed (DiMarco et al., 1981; Yambayamba et al., 1996). It is possible that calves weaned onto pasture as in the present study, require greater than 14 days to re-establish their pre-weaning NEFA and ketone status.

The lack of change in body weight during the course of the present study indicates that calves did not lose weight in the first day after weaning regardless of the distance walked by the calves. Results from previous studies evaluating alternative weaning strategies and effects on body weight during the first week following weaning are equivocal. The changes in calf body weight range from: 1) body weight loss in the first week after abrupt weaning with no other treatment impositions occurring and when there was inclusion of a trainer cow with calves (Loerch and Fluharty, 2000; Gibb et al., 2000; Haley et al., 2005), 2) to increased body weight in the first week after calves were weaned using a two-stage protocol (Haley et al., 2005); and 3) to increased body weight when using a fence-line approach (Campistol et al., 2013) for weaning.

Values for calf chute scores and exit velocity in the present study were not different among treatments. Temperament scores decrease as the number of times an animal goes through a working facility increases (Curley et al., 2006). The decrease in temperament score depends on the genetic predisposition of the animal and previous experiences with being worked with at the handling facility (Grandin and Shivley (2015).

In summary, calves placed with a familiar social facilitator animal on pasture at weaning walked less and devoted less time to walking on the first day after weaning compared with control calves. There were no differences in values for this variable between treatment groups on day 7 or 14, indicating that calves were acclimated to the weaning process by these times post-weaning. There were no observable differences in body weight or average daily gain, though the placement of the social facilitator animal with calves resulted in a decrease in the total distance calves walked on day 0. We believe the positive effects of placement of the social facilitator animal with calves on distance and time calves devoted to walking is due to allelomimicry, in which calves modelled their behaviors after those of the social facilitator animal. It is speculated that the familiarity of the social facilitator animal with the calves also contributed to this effect. The results from the present study indicate that while calf body weight was not affected by the placement of a social facilitator with calves at weaning, calf welfare was improved. This is important, as industry welfare standards are continually evolving, and consumers are becoming increasingly aware of the welfare states of animals used for food production. Thus, further research is warranted in this area to determine if the use of a familiar social facilitator animal at weaning is a beneficial management strategy from a beef production standpoint. Future research should also consider more frequent behavior sampling during the first 3 days after weaning to clarify what changes are occurring behaviorally and physiologically.

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Table 1. Starter diet composition

Ingredient	**% DM basis**
Ground corn	52.786
Soybean meal	3.050
Low fat dried distillers grain	18.010
Corn gluten feed	20.638
Limestone	1.15
Trace mineral salt	0.442
Vitamin A	0.062
Vitamin D	0.062
Vitamin E	0.019
Selenium (Vita Vet)	0.034
Amaferm (64 g/ lb concentrate)	0.767
A-V fat	2.980

Table 2. Behavioral ethogram for observations

Behavior:	**Description:**
Walking	All four legs moving with head raised in a non-feeding position
Vocalizing	Sound emitted from the calf and heard by the observer
Grazing/Feeding	Calf is either at the bunk or grazing with its head down, while actively engaged in consuming and masticating feed/grass; can include forward movement, but it is clear that the calf is in the process of grazing or feeding
Standing	All four legs fixed in place and immobile, calf is standing, but not participating in any other activity (i.e. walking, grazing, drinking, vocalizing)
Lying/Resting	Calf is lying down on all four legs in a resting position
Drinking	Head is near the water source and calf is actively engaged in drinking with its head down

Table 3. Mean (±SEM) for distance walked, time devoted to walking, area utilization index, average walking speed, and mean relative behavior counts for the social facilitator cows at weaning on day 0 and on days 7, and 14 after weaning.

	D 0	D 7	D 14
GPS Collar Data			
Distance, km	6.01 ± 1.57	3.97 ± 0.65	3.00 ± 0.34
Walking time[1], h	1.88 ± 0.49	1.23 ± 0.26	0.90 ± 0.11
Non-walking time, h	22.28 ± 0.45	22.90 ± 0.28	23.23 ± 0.10
AUI[2]	1.00 ± 0.05	0.83 ± 0.11	0.72 ± 0.14
Average speed, m/s	0.88 ± 0.04	0.91 ± 0.04	0.93 ± 0.02
Observed Behavior			
Walking	0.04	0.01	0
Vocalizing	0.03	0	0
Grazing	0.48	0.41	0.56
Standing	0.40	0.49	0.29
Lying	0.03	0.08	0.14
Drinking	0.02	0.01	0.01

[1]Walking time, h = Total time devoted to walking in 24 hours
[2]Area utilization index = Total polygonal area of the weaning paddock utilized by the social facilitator animal divided by the total area of the paddock

Table 4. Mean (±SEM) distance walked, time devoted walking, pasture utilization index and average walking speed for calves weaned with or without a social facilitator animal at weaning on day 0 and on days 7, and 14 after weaning.

n[1]	**Social Facilitator**			**Control**			**P-Values**		
	D0	D7	D14	D0	D7	D14	Trt	Day	Trt × Day
Distance, km	7.19 ± 0.79	2.99 ± 0.25	2.27 ± 0.25	9.02 ± 0.79	2 .80 ± 0.25	2.58 ± 0.25	0.08	<0.01	<0.01
Walking time[2], h	2.46 ± 0.28	0.93 ± 0.13	0.65 ± 0.12	3.14 ± 0.28	0.84 ± 0.13	0.78 ± 0.12	0.05	<0.01	<0.01
Non-walking, h	21.71 ± 0.28	23.22 ± 0.09	23.47 ± 0.08	21.00 ± 0.28	23.26 ± 0.09	23.36 ± 0.08	0.04	<0.01	0.05
AUI[3]	0.98 ± 0.05	0.75 ± 0.05	0.56 ± 0.05	0.97 ± 0.05	0.75 ± 0.05	0.69 ± 0.05	0.10	<0.01	0.07
Average speed, m/s	0.82 ± 0.01	0.92 ± 0.02	1.03 ± 0.03	0.81 ± 0.01	0.92 ± 0.02	0.99 ± 0.03	0.20	<0.01	0.55

[1]n = 4 replicates of 10 heifers per treatment

[2]Walking time, h = Total time devoted to walking in 24 hours

[3]Area Utilization Index = Total polygonal area of the weaning paddock utilized by the calf divided by the total area of the paddock

Table 5. Mean (±SEM) relative behavior counts, chute score and exit velocity for calves weaned with or without a social facilitator at weaning on day 0 and on days 7, and 14 after weaning.

n[1]	**Social Facilitator**			**Control**			**SEM**	**P-Values**		
	D0	D7	D14	D0	D7	D14		Trt	Day	Trt × Day
Observed Behavior										
Walking	0.06	0	0	0.15	0	0	0.019	0.07	<0.01	0.04
Vocalizing	0.17	0	0	0.21	0	0	0.032	0.61	<0.01	0.77
Grazing	0.42	0.60	0.58	0.25	0.51	0.45	0.108	0.16	0.09	0.93
Standing	0.25	0.32	0.27	0.32	0.35	0.34	0.098	0.42	0.80	0.96
Lying	0.07	0.07	0.12	0.05	0.12	0.18	0.045	0.45	0.13	0.60
Drinking	0.03	0.01	0.03	0.02	0.02	0.03	0.016	1.00	0.70	0.95
Temperament data										
Chute score[2]	1.94	1.85	1.85	1.82	1.65	1.54	0.153	0.15	0.29	0.72
Exit velocity[3], s	1.12	0.79	0.71	1.07	1.17	0.75	0.310	0.19	<0.01	0.26

[1]n = 4 replicates of 10 heifers per treatment
[2]Chute score = Score of 1 to 5 (1 = calm, no movement; 2 = restless, shifting; 3 = squirming, occasionally shaking of the chute; 4 = continuous vigorous movement and shaking of the chute; 5 = rearing, twisting of the body, or violent struggling)
[3]Exit velocity, s = the amount of time in seconds that it takes a calf to travel 1.8 meters after exiting the chute

Table 6. Mean (±SEM) body weight, average daily gain, non-esterified fatty acid and β-hydroxybutyrate plasma concentrations for calves weaned with or without a social facilitator at weaning on day 0 and on days 7, and 14 after weaning.

n[1]	Social Facilitator				Control				SEM	P-Values		
	D0	D1	D7	D14	D0	D1	D7	D14		Trt	Day	Trt × Day
Body weight, kg	182.3	183.0	185.5	189.9	182.3	177.6	185.9	191.1	9.53	0.75	<0.01	0.15
ADG, kg/d	---	---	0.43[2]	0.59[3]	---	---	0.48[2]	0.73[3]	0.26	0.58	0.38	0.96
NEFA, μEq/L	222.6	377.2	451.6	295.9	221.9	426.7	357.4	166.3	52.25	0.03	<0.01	0.01
β-hydroxybutyrate, μmol/L	200.6	269.5	323.2	267.2	193.0	278.6	279.6	279.7	23.48	0.45	<0.01	0.04

[1]n = 4 replicates of 10 heifers per treatment
[2]Average daily gain (kg/d) from day 0-7
[3]Average daily gain (kg/d) from day 7-14

Chapter 3. Intranasal oxytocin alters non-esterified fatty acid mobilization in abruptly weaned beef calves placed on pasture

Abstract

The purpose of this study was to evaluate the hypothesis that intranasal oxytocin provided at weaning (day 0) would decrease weaning distress behaviors such as walking and thus have a positive effect on body weight and plasma metabolites. A total of twenty Simmental/Angus crossbred calves were randomly assigned to either the intranasal oxytocin (**OXT**) treatment or the intranasal saline (**CON**) treatment. All calves were only given their respective intranasal treatment on the day of weaning (d 0), and then placed on pasture together. Calves were weighed and blood sampled on days 0, 1, 7, and 14 post-weaning. Blood samples were subsequently used to quantify non-esterified fatty acids, β-hydroxybutyrate, and cortisol. All calves in both treatment groups were fitted with an individual global positioning system collar that recorded calf location every ten seconds for a 24 hour period on days 0, 7, and 14 to quantify and evaluate walking behaviors. Additionally, behavioral observations were made on days 0, 7, and 14 with instantaneous sampling from 0730 to 0830, 1200 to 1300, and 1700 to 1800 h. To further evaluate calf behavior, chute scores and exit velocities were recorded on days 0, 7, and 14. There were no treatment or treatment × day effects ($P > 0.05$) for the GPS collar parameters (distance, walking time, non-walking time, area utilization index, average speed), behavioral observations, body weight, average daily gain, chute score, exit velocity, β-hydroxybutyrate, or cortisol concentrations, however, there was a day effect

($P < 0.05$) for these parameters. Treatment did influence NEFA concentrations, with the CON calves having a greater plasma concentration on day 1 compared with OXT calves ($P = 0.02$). These data imply that OXT calves may have had altered metabolic responses immediately after weaning that could have altered NEFA concentrations, but this change was not substantial enough to impact body weights or walking behaviors.

Key Words: behavior, global positioning system, stress response, welfare

Introduction

The bond between a cow and her calf is a mutual, affectionate, and long-lasting social investment (Newberry and Swanson, 2001). When this bond is discontinued at weaning, abnormal stress behaviors such as locomotion and vocal signaling are exhibited by both the mother and offspring (Panskepp, 1998). Stress and anxiety experienced by calves during the weaning period are some of the most critical factors that negatively impact growth performance (Campistol, 2010). Though it is stressful for both cows and calves, weaning decreases the nutritional and production requirements of the cow; therefore, improving her body weight and reproductive success (Enríquez et al., 2011).

The hypothalamic-pituitary-adrenal axis (HPA) and sympathetic adrenomedullary system (SAM) work synergistically and are responsible for initiating a response in the body when an animal experiences a stressor (Burdick et al., 2011). Oxytocin has been observed to be released in response to stress; therefore, it is hypothesized that the hormone is potentially involved in the attenuation of the HPA axis (Tilbrook and Clarke, 2006). Although literature concerning the endogenous release of oxytocin in stressful environments is limited, Sutherland and Tops (2014) reported that when lactating dairy cows were exposed to a novel environment (new milking parlor), oxytocin concentrations initially decreased and then steadily increased over days of milking in the new parlor. The authors suggested that in this context, oxytocin played a part in habituation to psychological stressors such as novel environments. Another study performed with Holstein steers found that intracerebroventricularly infused oxytocin (50 µg) decreased plasma cortisol concentrations (Yayou et al., 2008). Preliminary findings in humans, rats, and more recently livestock, provide promising evidence that oxytocin can attenuate the stress response and decrease anxiety-related behaviors when animals are exposed to a stressor. Thus, the objective of the current study was to assess the impact of intranasally administered oxytocin on calf growth and behavioral responses during the weaning period. It was hypothesized that the intranasal oxytocin given at weaning would minimize the negative behaviors associated with weaning by decreasing total distance walked and time spent walking by calves, thus having a positive effect on calf body weight and metabolic status observed through decreased NEFA and β-hydroxybutyrate concentrations. It was also hypothesized that intranasal oxytocin would decrease calf anxiety which would be observed through decreased chute scores and slower exit velocities from the chute.

Materials and Methods

All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee (Animal Use Protocol #2018A00000085). The following study was completed in September 2018 at the Jackson Agricultural Research Station (JARS; Jackson, OH, USA).

Animals and Management

A total of 20 Angus x Simmental heifer calves (194 ± 7.4 kg, 162 ± 9 days of age) were used in the following weaning study. All calves were born and remained at JARS for the duration of the study. Before calves were weaned, they were maintained with their dams on tall fescue pastures. All calves were vaccinated with a modified live virus (Bovi-Shield Gold 5, Zoetis Services LLC., Parsippany, NJ, USA) against IBR, BVD (Types 1 and 2), PI_3, and BRSV viruses, and a bacterin-toxoid for prevention of bovine pneumonia (One Shot, Zoetis Services LLC., Parsippany, NJ, USA). All 20 calves were also de-wormed at the time of vaccination.

On the day of weaning (d 0), the 20 heifer calves were randomly allotted to one of two treatment groups, intranasal oxytocin or saline (OXT, CON, Isotonic saline 0.9%, Oxytocin, 20 IU/mL Vetone®, Bimeda-MTC Animal Health Inc, Ontario, Canada). Treatments were only given on the day of weaning (day 0). Dose rates for the oxytocin were 0.6 IU/kg BW. Dose rates for the saline treatment were applied in the same manner as the oxytocin to keep dosing amounts similar for both treatments. Doses ranged from 5.5 to 7 mL, dependent upon individual calf body weight. Half of each dose was administered into each nostril with a mucosal atomization device (Nasal™ Teleflex® Inc., Morrisville, NC). After receiving their treatments, all calves were housed together in the same pasture. Due to weather and forage conditions, all calves were moved when necessary to a new pasture to allow for adequate forage. From days 0 to 6 calves were placed in a 1 hectare pasture, on days 7 to 9 calves were moved to a 0.4 hectare pasture, and on days 10 to 15 calves were moved to a 1.3 hectare pasture. Calves were provided 0.91 $kg \cdot animal^{-1} \cdot day^{-1}$ of a 17% protein starter diet (Table 7).

Global Positioning System Collars

All calves in each treatment (OXT, CON) had a collar placed around their neck with a global positioning system (GPS) tracking device at weaning on day 0. The collars were on the calves for 24 hours on days 0, 7, and 14 and were subsequently removed on days 1, 8, and 15 to perform battery recharging. The GPS devices were set to receive a

GPS position of the calves every 10 seconds. The data extracted from the collars were total distance travelled (km), walking time (h, total time spent strictly on walking), non-walking time (h, total time spent on all other activities besides walking), area utilization index (AUI-calculated by determining the area that each individual calf was tracked on days 0, 7 and 14 and dividing this area by the total area of the pasture), and average speed (m/s). This data were obtained using the Garmin BaseCamp software (Garmin International, Inc., Olathe, KS).

The collars used were the Garmin Astro 430/T5 GPS/GLONASS Dog Tracking System. The collars were reported by Garmin to be accurate to within 15 meters 95% of the time when there were no physical obstructions of the system to the celestial sphere. Garmin also stated that "Generally, users will see accuracy within 5-10 meters under normal conditions."

Behavioral Observations

Behavioral observations were recorded using an ethogram (Table 8) on days 0, 7, and 14. Observations were recorded on days 0, 7, and 14 from 0730 to 0830, 1200 to 1300, and 1700 to 1800 h with an instantaneous sampling technique (Lehner, 1979) every 10 minutes throughout the duration of the hour (seven total scan samples/hour) to provide observations in the morning, afternoon, and evening. For analysis, behaviors for an entire day of scan sampling for each individual calf were added and then divided by the total number of counts that day to provide a relative count of how many times a calf was expressing certain behaviors during the observation periods for each treatment group. For example, if there were 10 counts recorded for an individual calf feeding during the three hours of observations, the relative count would be calculated as follows:

$$\frac{(10 \text{ recorded counts})}{\left(7\,\frac{\text{scan samples}}{\text{h}} \times 3\text{ h}\right)} = \frac{10}{21} = 0.48$$

Sampling and Analysis

Heifer calves were abruptly weaned from their mothers on day 0 when they received their intranasal treatment. All calves were blood sampled (10 mL) via jugular venipuncture and body weight was quantified on days 0, 1, 7, and 14. The blood samples were collected in vacutainer tubes containing lithium heparin. The samples were then placed on ice and transferred back to the laboratory where the tubes were centrifuged for 25 minutes at approximately 1,800 *x g* and 4°C. The plasma was then further aliquoted into individual microcentrifuge tubes to determine non-esterified fatty acid (NEFA), β-hydroxybutyrate (β-HB), and cortisol concentrations. Plasma NEFA concentrations were determined using micro titer plates and a plate reader in a 2-reaction, enzyme-based assay (NEFA-HR(2), FujifilmWako Diagnostics, USA, Richmond, VA) as previously described by Johnson and Peters (2003). The inter-assay variation was 5.4% and intra-assay variation was 4.8%. β-hydroxybutyrate concentrations were quantified using LiquiColor procedure (Beta-Hydroxybutyrate LiquiColor Test Endpoint, Stanbio Laboratory, Boerne, TX). Inter-assay variation was 6.0%, and intra-assay variation was 7.5%. Cortisol concentrations were quantified using a commercially available radioimmunoassay kit (MP Biomedicals, LLC., Solon, OH). This assay was validated in using bovine plasma, and the protocol was modified by using 50 µl of plasma, rather than 25 µl. The minimum level of detection was 5 ng/mL. The inter-assay variation was 10.6%, and intra-assay variation was 5.8%, respectively.

Chute Score and Exit Velocity

To evaluate behavior, chute scores and exit velocity were recorded on days 0, 7, and 14. A method previously described by Grandin (1993) was followed to visually determine chute scores. This system is based on a range of 1 to 5 while calves are restrained in the head chute: 1 = calm, no movement; 2 = restless, shifting; 3 = squirming, occasional shaking of the chute; 4 = continuous vigorous movement and shaking of the chute; 5 = rearing, twisting of the body, or violent struggling. Exit velocities were recorded with the use of infrared sensors (FarmTek, Inc., Wylie, TX) and a method previously described by Burrow et al. (1988) in which the "start" sensor was placed 0.6 meters from the head gate of the chute system, and the "finish" sensor was placed 1.83 meters from the start sensor. The exit velocity was recorded as the amount of time it took for each calf to travel the 1.83 meters, and the exit velocity was expressed in seconds.

Statistical Analysis

Calf body weight, average daily gain, NEFA concentration, β-hydroxybutyrate concentration, cortisol concentration, chute scores, exit velocities, and calf collar data (distance, walking time, non-walking time, area utilization index, average speed) were all analyzed as a completely randomized design with repeated measures using the proc MIXED procedure of SAS (9.4, SAS Inst. Inc., Cary, NC). The model included treatment, day, and their interaction as fixed effects, and calf as the random effect. Analyses for all models were conducted using different covariance structures and the model with the lowest AIC was selected. Differences were considered to have resulted from the treatments if $P \leq 0.05$. Tendencies were considered to have resulted from the treatments if $P \leq 0.1$ and > 0.05.

Results

Global Positioning System Collar Data

The data obtained from the Garmin collars are presented in Table 9. Not all collars recorded for a full 24 hours on days 0, 7, or 14, therefore the data presented is representative of 16 hours (2300 to 1500 h) that each collar did record on all three sample days. Therefore, the walking time and non-walking time sum to 16 hours on all three sample days. There was a day effect for all collar parameters ($P < 0.01$) including distance walked, walking time, non-walking time, area utilization index (AUI), and average speed, however, there were no treatment or treatment × day effects observed for distance walked, walking time, non-walking time, or area utilization ($P > 0.05$). There was a tendency toward a treatment × day effect ($P = 0.07$) for average speed with both treatment groups' average speed increasing with each subsequent sampling day.

Behavior Observations

The mean relative behavior counts from the 3 days of visual observations (days 0, 7, and 14) are presented in Table 10. There was a day ($P < 0.05$) effect for all behaviors that were observed (walking, vocalizing, grazing, standing, lying, drinking), however, there were no significant treatment or treatment × day effects ($P > 0.05$). Calves in both treatment groups were only observed walking and vocalizing on day 1. For both treatments, grazing was least on day 0, and greatest on day 7. Standing was observed to be least on day 7 and greatest on day 14 in both treatments. Lying and drinking behaviors were observed to be relatively constant over the observation period.

Chute Score and Exit Velocity

The chute score and exit velocity data are presented in Table 10. There were no treatment, day, or treatment × day effects for chute score ($P > 0.05$). There was, however, a day ($P < 0.01$) effect for exit velocity, with both treatment groups having the slowest exit velocity on day 0, and exit velocity getting faster with each subsequent sampling day.

Performance and Plasma Metabolites

The body weight, average daily gain, NEFA, β-hydroxybutyrate, and cortisol plasma concentration data is presented in Table 11. There were no treatment or treatment × day effects on body weight or average daily gain, however, there was a day effect for body weight and ADG ($P < 0.01$, $P < 0.01$, respectively). Both treatments lost weight on day 1, and then gained weight on days 7 and 14. All calves had similar average daily gains from day 0-7 and day 7-14, having greater gains in the second week after weaning. There was a treatment ($P = 0.02$) and day ($P < 0.01$) effect on NEFA concentrations with the CON group having a greater concentration compared with the OXT group on day 1 (644.1 compared with 416.6 ± 80.04 μEq/L), day 7 (568.9 compared with 449.0 ± 80.04 μEq/L), and day 14 (586.1 compared with 407.2 ± 80.04 μEq/L). There was a day ($P < 0.01$) effect observed for β-hydroxybutyrate concentration with both treatment groups' concentrations increasing from day 1 to day 14. There were no treatment or treatment × day effects on cortisol, however, there was a day effect ($P < 0.01$) with both treatment groups having greater cortisol concentrations on day 1 compared with days 0, 7 and 14.

Discussion

The current results do not support the hypothesis that intranasal oxytocin administered on the day of weaning would decrease the distance walked and time devoted to walking by calves when all calves are placed together on pasture. It is possible that there were no effects observed in any of the collar data evaluated (distance, walking time, non-walking time, area utilization index, and average speed) or behaviors observed because the two treatment groups were housed together in the same pasture, and may have synchronized their behaviors. The behavioral observations made in this experiment were very similar among both treatment groups, also indicating a degree of synchrony among the calves. It is important to note that these are relative behavior counts, and do not reflect the behavioral patterns of the entire day, rather just the three hours that the two treatment groups were observed on days 0, 7 and 14. Behavior observations recorded during three 1-hour periods (morning, noon, and evening) were considered sufficient to capture relative behavior counts in the present experiment since daily grazing and activity cycles in cattle tend to be very consistent, with major grazing events occurring in the morning from 0400 h to 0800 h, and in the evening from 1800 h to 2100 h, and minor grazing events occurring from 0100 h to 0200 h and around 1200 h (Lyons and Machen, 2000).

Calves in the OXT and CON groups walked 9.12 and 9.58 ± 0.141 km on day 0, respectively. Distances for both treatment groups on days 7 and 14 from the present study, however, were comparable to distances reported in Chapter 2. In pasture systems with adequate forage and temperate climates, cattle will typically walk 4 to 8 km each day (Squires, 1981). In moderate and extreme climatic environments cattle have been recorded to walk 7 to 14 km/d (Hodder and Low, 1978) and even up to 20 km/d (Squires, 1981) to find forage. Therefore, while the distances reported in the current study on day 0 are greater than previously reported for abruptly weaned calves placed on pasture with or without a social facilitator, these distances are still within reason.

Observations of calf behavior on day 0 indicate that calves performed minimal grazing bouts, and increased walking and standing bouts. Walking bouts were observed to be 0.28 ± 0.014 on day 0, and no walking bouts were observed on days 7 or 14. Grazing was observed to be greatest on day 7 (0.67 ± 0.019) and decreased on day 14 (0.39 ± 0.019). It is possible that temperature-humidity index (THI; Figure 1) and the resulting heat stress may have affected calf behavior such that behavior shifted from grazing to standing and walking, especially on day 0 when calves were weaned. *Bos taurus* cattle exhibit mild heat loads when the THI exceeds 72 (Du Preez et al., 1990; Armstrong, 1994) and exhibit severe head loads when the THI is around 79 (Hahn and Mader, 1997). The average maximum THI throughout the duration of the present study was 77.6, and the average minimum was 63.2. Therefore, the mild to severe THI during the study may have acted as an additional stressor and may explain why calves grazing time was negligible on day 0 but walking time and walking distances were increased on day 0.

The present results do support the hypothesis that intranasal oxytocin administered at weaning would decrease NEFA concentrations. Both treatments had similar NEFA concentrations on day 0, as expected since blood samples were obtained at separation and until then calves had all been nursing their dams. Initial NEFA concentrations obtained on day 0 are comparable to NEFA concentrations reported in Chapter 2 on the day of maternal separation. While both treatment groups had elevated NEFA concentrations on day 1, the CON group had greater concentrations and this difference remained throughout the remainder of the study. We believe this difference in plasma NEFA concentrations is related directly to feed intake, as the calves walked the same distance, had very similar behavior counts on the first day of weaning, and had no significant differences in plasma cortisol concentrations. This indicates that the release of NEFA was not dependent upon distance walked or the hypersecretion of cortisol, as this would cause calves to mobilize body stores.

Previous data by Rault et al. (2011) reported increased aggression and agonistic interaction such as parallel and head pushing behaviors in pigs treated repeatedly with intranasal oxytocin at 1, 2, and 3 days of age. These results lead us to believe that calves treated with intranasal oxytocin on the day of weaning could have exhibited more aggressive and agonistic behaviors when all calves were offered the starter diet in the feed bunk. If OXT calves were more aggressive and consumed more feed on the day of weaning, this could have alleviated the need to mobilize as much NEFA as the CON calves that consumed less feed.

In summary, calves given intranasal oxytocin at weaning did not experience any behavioral changes related to walking distances or time devoted to walking. It is possible that the behavioral synchrony previously observed in cattle may have been simply more prevalent than differences due to the administration of oxytocin. Body weight, average daily gain, chute scores, exit velocities, β-hydroxybutyrate and cortisol concentrations were also not impacted by intranasal treatment, however, NEFA concentrations were greatly elevated in the CON treatment compared with the OXT treatment on days 1, 7, and 14 after weaning. While the mechanism remains unknown, the current results indicate that oxytocin is capable of minimizing the increased NEFA concentrations observed during the first several days after weaning. Thus, further research is warranted to determine oxytocin's mode of action in reducing NEFA concentrations, and if the administration of oxytocin is capable of inducing significant enough changes to impact production.

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Table 7. Starter diet composition

Ingredient	**% DM basis**
Ground corn	52.786
Soybean meal	3.050
Low fat dried distillers grain	18.010
Corn gluten feed	20.638
Limestone	1.15
Trace mineral salt	0.442
Vitamin A	0.062
Vitamin D	0.062
Vitamin E	0.019
Selenium (Vita Vet)	0.034
Amaferm (64 g/ lb concentrate)	0.767
A-V fat	2.980

Table 8. Behavioral ethogram for observations

Behavior:	Description:
Walking	All four legs moving with head raised in a non-feeding position
Vocalizing	Sound emitted from the calf and heard by the observer
Grazing/Feeding	Calf is either at the bunk or grazing with its head down, while actively engaged in consuming and masticating feed/grass; can include forward movement, but it is clear that the calf is in the process of grazing or feeding
Standing	All four legs fixed in place and immobile, calf is standing, but not participating in any other activity (i.e. walking, grazing, drinking, vocalizing)
Lying/Resting	Calf is lying down on all four legs in a resting position
Drinking	Head is near the water source and calf is actively engaged in drinking with its head down

Table 9. Mean (±SEM) for distance walked, time devoted to walking, area utilization index and average walking speed for calves weaned with intranasal oxytocin or intranasal saline on day 0, 7, and 14 after weaning.

n[1]	Oxytocin			Saline			SEM	P-Values		
	D0	D7	D14	D0	D7	D14		Trt	Day	Trt × Day
Distance, km	9.12	2.69	1.85	9.58	2.78	1.90	0.141	0.55	<0.01	0.89
Walking time[2], h	3.07	0.86	0.52	3.19	0.88	0.55	0.126	0.65	<0.01	0.80
Non-walking, h	12.93	15.14	15.48	12.81	15.12	15.45	0.140	0.39	<0.01	0.88
AUI[3]	0.64	0.85	0.34	0.68	0.87	0.34	0.058	0.53	<0.01	0.81
Average speed, m/s	0.82	0.88	0.96	0.83	0.91	0.93	0.015	0.53	<0.01	0.07

[1]n = 10 heifers per treatment

[2]Walking time, h = Total time spent walking. Walking time and non-walking time sum to 16 hours of total GPS data for days 0, 7, and 14.

[3]Area Utilization Index = The total polygonal area of the weaning paddock utilized by the calf divided by the total area of the paddock

Table 10. Mean (±SEM) for relative behavior counts, chute score and exit velocity for calves weaned with intranasal oxytocin or intranasal saline on day 0, 7, and 14 after weaning.

n[1]	Oxytocin			Saline			SEM	P-Values		
	D0	D7	D14	D0	D7	D14		Trt	Day	Trt × Day
Observed Behavior										
Walking	0.28	0	0	0.28	0	0	0.014	0.91	<0.01	0.99
Vocalizing	0.15	0	0	0.14	0	0	0.020	0.94	<0.01	0.99
Grazing	0.06	0.67	0.36	0.08	0.64	0.39	0.019	0.70	<0.01	0.26
Standing	0.28	0.22	0.44	0.30	0.22	0.44	0.027	0.77	<0.01	0.97
Lying	0.23	0.08	0.19	0.19	0.11	0.14	0.028	0.52	<0.01	0.19
Drinking	0	0.03	0.01	0.01	0.03	0.03	0.009	0.38	0.02	0.79
Temperament Data										
Chute Score[2]	1.7	1.4	1.4	1.3	1.4	1.2	0.188	0.33	0.41	0.41
Exit Velocity[3], s	1.7	1.3	1.0	1.4	1.0	0.8	0.279	0.27	<0.01	0.58

[1]n = 10 heifers per treatment

[2]Chute score = Score of 1 to 5 (1 = calm, no movement; 2 = restless, shifting; 3 = squirming, occasionally shaking of the chute; 4 = continuous vigorous movement and shaking of the chute; 5 = rearing, twisting of the body, or violent struggling)

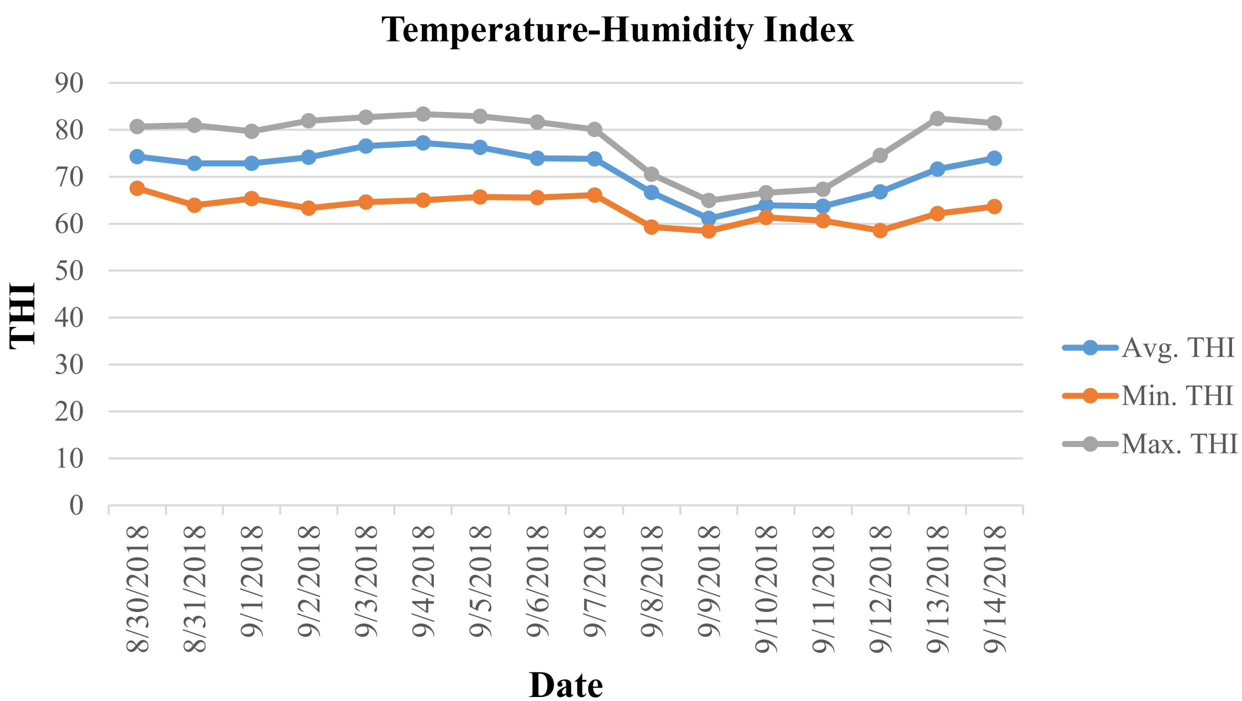
[3]Exit velocity, s = the amount of time in seconds that it takes a calf to travel 1.8 meters after exiting the chute

Table 11. Mean (±SEM) for body weight, average daily gain, non-esterified fatty acid, β-hydroxybutyrate, and cortisol plasma concentrations for calves weaned with intranasal oxytocin or intranasal saline on days 0, 1, 7, and 14 after weaning.

n[1]	**Oxytocin**				**Saline**				**SEM**	**P-Values**		
	D0	D1	D7	D14	D0	D1	D7	D14		Trt	Day	Trt × Day
Body weight, kg	194.4	184.9	197.2	202.1	193.0	182.3	196.7	200.3	2.83	0.65	<0.01	0.83
ADG, kg/d	---	---	0.40[2]	1.54[3]	---	---	0.53[2]	1.14[3]	0.268	0.70	<0.01	0.54
NEFA, µEq/L	258.1	416.6	449.0	407.2	239.1	644.1	568.9	586.1	80.04	0.02	<0.01	0.09
β-hydroxybutyrate, µmol/L	183.1	262.0	305.1	308.3	153.2	286.4	316.5	334.9	28.60	0.60	<0.01	0.27
Cortisol, ng/mL	10.0	12.7	10.0	10.0	11.3	11.4	10.0	10.0	0.68	0.99	<0.01	0.32

[1]n = 10 heifers per treatment
[2]Average daily gain (kg/d) from day 0-7
[3] Average daily gain (kg/d) from day 7-14

Figure 1. Temperature-Humidity Index over the 15 day period of the weaning study.



Chapter 4. General Discussion

Weaning is stressful for both cows and calves and causes the expression of abnormal behaviors due to social separation and cessation of milk to the calf. Although it is stressful for both cows and calves, weaning at 5-8 months of age is considered necessary as it decreases the cow's nutritional and production requirements (Enríquez et al., 2011). Stress and anxiety experienced by calves during the weaning period are some of the most critical factors that negatively impact growth performance (Campistol, 2010). This presents a concern to the beef industry as modern consumers are increasingly more conscious of on-farm animal welfare (Frewer et al., 2005) and food production processes (Olynk, 2010). It is important as an industry to acknowledge the need to improve welfare standards during weaning, and to minimize production losses during the weaning period. While alternative weaning strategies such as two-stage, fence-line, and the use of a trainer cow have been proposed, the literature provides equivocal results on the efficacy of these methods.

In the alternative weaning method outlined in Chapter 2, calves were placed with a familiar social facilitator on pasture at weaning. The calves placed with the social facilitator walked less and devoted less time to walking on the day of weaning (day 0) compared to control calves. These differences were not seen on day 7 or 14 after weaning, indicating that calves were acclimated to the weaning period and the typical exploratory behaviors that were performed on day 0 were minimal once calves became familiar with their environment. Though the social facilitator calves walked fewer total kilometers than control calves on the day of weaning, there were no observable differences in body weight or average daily gain. Calves in the social facilitator group had similar walking distances when compared with the social facilitator cow on all 3 days they were recorded. This provides indication that placing calves with a familiar social facilitator induced a degree of allelomimicry, in which the calves modelled their own behavior after the social facilitator cow. While we did not detect any differences in body weight, it is apparent that calves walked less, and this is suggestive of improved calf welfare during the weaning period. Thus, further research in this area is warranted to determine if the use of a familiar social facilitator at weaning can be beneficial on growth parameters of calves. Future research should also consider the familiarization of the social facilitator cow with the pasture before the day of weaning to minimize her own exploratory behaviors of the new environment. Based on the results of the present experiment, future research should also consider focusing on behavior and production differences in the first 72 hours after weaning, as calves in both treatment groups seemed to be accustomed to the weaning period by day 7.

Chapter 3 evaluated the use of intranasal oxytocin at weaning as a potential pharmacological agent capable of attenuating the stress associated with weaning. Oxytocin has been observed in some situations to be released in response to severe stress; therefore, it is hypothesized that the hormone is potentially involved in the attenuation of the HPA axis (Tilbrook and Clarke, 2006). The present experiment, however, did not support the hypothesis that intranasal oxytocin would attenuate the stress response and therefore calves would walk less, devote less time to walking, and would have improved plasma metabolite status. Oxytocin may not have the capability to combat weaning stress as it was exogenously administered only on the day of weaning. It is difficult to speculate what would have happened in the present experiment if intranasal oxytocin was given to calves on a daily basis, however, this is not a realistic production practice, thus oxytocin was only administered on the day of weaning. It is also possible that as the oxytocin and saline treated calves were placed in a single pasture together at weaning, the calves synchronized their behaviors and acted as a cohesive group, therefore, there were no observable differences in calf GPS or behavioral data. Future research utilizing oxytocin as an agent to reduce stress in beef calves at weaning should consider the repeated administration of the neuropeptide during the first 72 hours after weaning, as the present experiment only administered oxytocin on the day of weaning.

Subsequent weaning research in beef calves should further investigate methods to reduce stress, and thus stress behaviors exhibited by cows and calves during the first several days after weaning. Future research using a social facilitator at weaning should work to find ways to further reduce the distances calves walk, such that there will be observable differences in growth. Behavior, growth, and energy balance parameters should be observed more frequently during the first several days after weaning in order to gain a better understanding of weaning stress effects and how alternative weaning methods such as the use of a social facilitator alter these effects. Further steps in oxytocin research should work to further distinguish how and where it is having its effect on the

HPA axis specifically in beef cattle, and to determine under what circumstances oxytocin will be beneficial in our beef production system.

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