

**The Effects of Pair versus Individual Housing Preweaned Dairy Calves on
Behavior, Growth, and Acquired Immunity**

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**Clay Brent Kesterson
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DEDICATION

I would like to thank the University of Tennessee, graduate students that have become close friends, the staff at ETREC, family, and my supportive fiancé for giving the moral support to finish my MS degree.

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I thought as an 8-year-old boy watching *Babe*, *Bambi*, *Anabel's Wish*, and most importantly *The Crocodile Hunter*, they were preparing me to be the greatest vet possible. I also wanted the accomplishment of being the first doctor in my family. However, when I was introduced to the Department of Animal Science at WSCC and UTK, my eyes opened to the multiple careers within animal science. Through my undergraduate career, I worked with cattle every week at a local community college, for Dr. Chambers at Morristown Animal Hospital, and for family friends. From then, I knew that I wanted to work with cows. I began working with graduate students, Randi Black and Jessie Kull in dairy cattle welfare. I knew after helping out with their research, I wanted my MS degree. So 2 years later, here I am defending my thesis! I know two things from this experience: I can do whatever I set my mind to, and I sure as heck do not want my Ph.D.

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ABSTRACT

Our objective was to determine the effect of pair versus individual housing of calves on growth, behavior, and immunity. At 5 ± 1 d relative to birth, calves with successful passive transfer of immunoglobulins from colostrum were blocked by sex and birth date. Housing treatment was assigned to either pair ($n = 14$) or individual ($n = 14$) housing. Calf pairing was implemented by combining two individual pens. One paired calf served as the focal calf and the other imposed treatment. All data were collected from the focal calf in pair housing. Control calves remained individually housed. ADG, DM, feed refusals, and growth at weaning to one-week-post weaning did not differ between housing treatments. Paired calves spent more time awake, and increased standing time. Individual calves projected their head out of the pen more frequently. Hunger and anticipation behaviors did not differ between housing treatments. These data suggest paired calves were more active during the milk feeding stage. The increased activity of pair housed calves may suggest improved welfare of preweaned calves. IgG and IgM ELISA units to keyhole limpet hemocyanin did not differ between housing treatments. IgG ELISA units at d 14 were lower than d 3, 28, and 35 in all calves. IgM ELISA units were highest at d 3, but continued to drop thereafter. The stimulation index for delayed hypersensitivity to *Candida albicans* did not differ between housing treatments. However, they did increase over time, and peaked at 24 and 48 h post *C. albicans* injections. Pain sensitivity did not differ between housing treatments, however calves increased sensitivity at 27, 51, and 75 h, relative to disbudding. Pair housing dairy calves during the preweaned period did not negatively affect the immune development of dairy calves or pain from disbudding. This suggests social housing calves early in life does not suppress the immune system, or increase pain sensitivity after disbudding.

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CHAPTER I

**THE EFFECTS OF SOCIAL HOUSING ON BEHAVIOR, IMMUNITY, AND
STRESS OF NEONATAL CALVES**

INTRODUCTION

It is important to note that improving the welfare of dairy calves early in life has become a focal point in research and industry leaders. For instance, greater milk allowance to dairy calves throughout the milk feeding phase improves weight gain post weaning and reduces hunger behaviors (De Paula Vieira et al., 2008; Rosenberger et al., 2016), and calves provided increased space between 6 to 9 weeks of age had better regulation of neutrophils and less basal cortisol secretion (Hulbert and Moisé, 2016). In addition, dairy calves given the opportunity to socialize earlier were easier to work with during restraint, played more often, and were more competitive post weaning (Duve et al., 2012).

Calf welfare research has significantly been influenced by public perception. Ventura et al. (2016) observed when naïve consumers of dairy farming visited an operation, their perception of calf rearing changed from a previous positive outlook to a negative one. A general purpose for the changed perception was the concern that calves were reared individually and not with the dam or conspecifics. Therefore, it is essential for management practices in the dairy industry to consider to calf welfare and the acceptance of consumers in order to see dairy consumption increase.

Social deprivation in the early life has previously demonstrated learning difficulties, abnormal social behaviors, neurobiological changes, and higher instances of fear in multiple species (Harlow, 1965; De Paula Vieira, 2010). In the dairy industry, roughly 70% of dairy calves in the U.S. are taken from their dams and housed individually (USDA, 2014). Although social housing has the potential to increase cognitive function in dairy calves, hesitation to social

housing still exists. Leading factors for hesitation are typically cross suckling (Nielsen et al., 2018) and the fear of spreading disease to multiple calves (Hulbert and Moisé, 2016).

Neonatal calves are born agammaglobulinemic, which increases their vulnerability for disease transmission. Therefore, calves depend on colostrum from the dam for immunoglobulin circulation (Chase et al., 2008). The influence of these maternal antibodies from colostrum are most effective for the first 3 weeks of life (Hulbert and Moisé, 2016). Around that time, the protection of maternal antibodies begins to decrease, and calves begin to acquire their own immune cells to environmental antigens. With this window of susceptibility, most producers wait until calves are 8 weeks old for social housing. However, emphasis on colostrum management has reduced outward health hazards of calves (USDA 1992, USDA 2014); therefore, more natural rearing can be considered, such as social housing.

Milk price is heavily involved in management decision for producers, however other strategies beyond price support farming decisions, such as labor, convenience, and peer performance (Bragg and Dalton, 2004). Individuals might argue social housing calves requires new building supplies, and may be a financial burden during low milk prices. However, Wormsbecher et al. (2017) confirmed that pairing calves together using resources already on farm (i.e., hutches) was a convenient management change that improved the welfare of calves (more natural social behaviors) without negatively affecting calf performance or financial loss. In a recent survey conducted by Sumner et al. (2018), farmers are willing to alter management practices when provided benchmarks set by peers. Therefore, further investigation of socially reared calves is needed to create benchmarks for preweaned dairy calves housed in groups. Once

benchmarks are established, and the benefits are clear from social housing, producers may be keen to social housing calves earlier in life.

Previously, Costa et al. (2016) summarized scientific publications of social isolation and its detrimental effects to calves on cognition, social support during stress, and the inconsistent results of outward health (scours and respiratory diseases) associated with social housing. However, the recent abridged literature of calf activity, immune competence, and pain sensitivity of group-housed animals has yet to be summarized. This review begins with a brief summarization of calf activity, immune competence, stress, pain, and how social housing impacts each of these. When applicable, suggestions for future research will be pronounced to bring insight on the gap of knowledge that exists between dairy calf behavior, immunity, and how group housing influences these variables.

CALF BEHAVIOR

Lying Behavior

Time spent lying is a common behavioral response studied in dairy cattle in various environments, and typically serves as an indicator of good or poor welfare (Krawczel et al., 2012; Black et al., 2017; Kull et al., 2017). In dairy calves, lying behavior has many interpretations (Table 1). For instance, calves in semi-natural environments reared by their dams spent up to 80% of their time laying and hiding in tall grasses (Lidfors, 1994). Similarly, conventional raised calves have been noted to rest up to 17- 19 h/d when on clean, dry bedding (Camiloti et al., 2012; Bonk et al., 2013). Lying behaviors are important for growing cattle, and when restricted, can reduce growth success (Mogensen et al., 1997; Hänninen et al., 2005).

Environmental and management conditions can alter the lying behaviors of calves, and interpretations are warranted. Calves reared outdoors during cold temperatures rested less on their sides, and more with their necks and legs tucked near their bodies (Hänninen et al., 2010). Regardless of indoor or outdoor rearing, calves should be provided with dry bedding. Calves provided with bedding at high dry matter had greater lying time than those exposed to wet bedding (Camiloti et al., 2012). Different bedding types however, (rice hulls, granite fitness, sand, long wheat straw, or wood shavings) have observed similar lying and standing behaviors regardless of bedding type, and may be more dependent on dry matter than it is floor surface type (Panivivat et al., 2004). Similarly, bedding surface (sand, gravel, or rubber mats) did not affect lying time, however the inability to control stable and house flies negatively impacted calf lying behaviors (Kurman, 2014). These data suggest although lying time may not differ with bedding type, but the management of the environment can reduce rest in young calves.

Milk allocation can also influence lying time. Calves provided ad libitum amounts of milk spent more time lying than calves given a restricted diet (De Paula Vieira et al., 2008), suggesting restricted calves were more hungry than those fed ad libitum. Although restricted milk reduced lying time, lying postures were unaffected regardless of milk allowance of individually housed calves (Borderas et al., 2009). Although increasing lying time typically has positive outcomes in dairy calves and cows, greater lying time is not always an indication of healthy calves. For instance, calves inoculated with *Mannheimia haemolytica* on average, spent more time lying, and more time lying on their right side in relation to control calves (Eberhart et al., 2017).

It is important to appreciate the difference in lying behavior calves experience that are subjective to age, lying surfaces, environment, and milk allowance. For instance, calves on average spend roughly 81% of their time lying the first week of life, and by six weeks of age, lying time reduces to 73% (Leadley, 2018). In addition, For up to 21 weeks of age, calves rest from the hours of 0800 until approximately 1000 and again at 1800 until around 0400 (Hänninen, 2007). In order for calves to reach their highest potential, the environment, management style, and the limitations within these variables must be understood. In addition, the effects of social housing on lying behaviors needs to be considered to determine if having social partners improves or reduces the quality of rest.

Lying Behavior of Socially Housed Calves

Social housing animals has the potential to improve the quality of sleep in multiple species, however less is known on how social housing affects lying behaviors in dairy calves. Prior to weaning, calves reared with their dam performed less sleeping bouts than calves fed from a teat or bucket with no teat, thus suggesting better quality rest is achieved when calves are with the dam, but it is not clear if group housing can have the same affect (Hänninen et al., 2008a). From the data that is available of socially housed calves, suggests calves reared in groups or individually during the milk feeding phase had similar lying times, however at 14 -21 weeks of age, paired calves rested for shorter bouts than individually housed calves (Hänninen, 2007). In relation to individual and social housing at an early age, lying time of calves at 5 or 6.5 weeks of age did not differ (Dellmeier et al., 1985; Chua et al., 2002). Furthermore, weaned calves housed in homogeneous age groups at weaning spent more time lying, were more explorative, and had greater ADG in relation to calves introduced to pens of various ages

(Færevik et al., 2010). These data suggest that social housing coupled with age can influence the lying behaviors observed in preweaned and weaned dairy calves. Regardless of various forms of social contact (auditory, visual, olfactory, or group), lying behaviors did not change, and all calves laid approximately 5.5 hours upright during a 24 hour observation period (Jensen and Larsen, 2014). In contrasts, cross-bred calves housed individually spent more time lying down than pair housed calves (Babu et al., 2004), thus suggesting paired calves spent more time exploring their environment or were interrupted by penmate. Social housing and restricted space in young calves has concluded to disrupt lying time of paired calves (Wilt, 1985; Le Neindre, 1993). Therefore, it is suggested negative effects may result in less lying time as well. In summary, interpretation of these data have many conclusions.

Group-housed calves may interrupt other resting calves, which may be dependent upon age and space, or it may encourage by social facilitation for calves to be more explorative or playful. In addition, the increased time individual calves spend lying down may be a coping mechanism when calves are ‘bored’ due to the lack of companionship. Currently, data is inconsistent in regards to social housing and lying behaviors during the milk-feeding phase, and scientific recommendations are still lacking for the amount of rest appropriate for growing calves.

Sleep in Neonatal Animals

Sleep is defined as being in a state of immobility with the ability for rapid reversibility, unlike in a comma or under anesthesia (Siegel, 2005). Sleep is important for regular bodily function, and when deprived, vital systems suffer, such as thermoregulation, energy balance, and immune function (Bonnet et al., 2005). Sleep is a part of the biological circadian rhythm that is

regulated by the pacemaker located in the suprachiasmatic nuclei (SCN) just above the hypothalamus. The synchronized function of the SCN is dependent upon diet, lighting, hormonal-feedback, activity, and social cues (Buijs et al., 2003). In relation to hormone secretion, the SCN regulates the release of circadian hormones, such as cortisol and growth hormone (GH). Growth hormone and glucocorticoid secretions, are released during sleep and is critical for growing mammals (Steiger, 2002). Young animals without the ability to release GH have been associated with negative growth affects (Mogensen et al., 1997). Therefore, it is important for housing conditions to be evaluated to determine which methods can provide calves with the best quality rest. For instance, indoor and outdoor temperature (Hänninen et al., 2010), space allowance (Wilson et al., 1999), lights (Weiguo and Phillips), weaning (Veissier et al.), and the presence of a partner (Babu et al., 2004) have all impacted dairy calf resting and sleep patterns.

Sleep in mammalian infants early in life is important for growth, development, and has beneficial outcomes later in life (Benington and Craig Heller, 1995). Sleep in young animals has been studied via electroencephalography and divided into 4 different stages: non-rapid eye movement (NREM) which is thought of as dreamless sleep; rapid eye movement (REM) which is considered dreaming or ‘twitching’ sleep; Transition sleep (T) is mostly noted in human infants and is considered to transitional sleep between wakefulness; wakefulness (W) is described as eyes open, head raised, irregular respiration, and vocalization (Hänninen et al., 2008a; Grigg-Damberger, 2016). The importance of sleep begins in utero where muscle twitching is linked to the organization of spinal sensorimotor circuits and trigger burst of electrical activity for developing the somatosensory cortex (Grigg-Damberger, 2016).

Postpartum, NREM sleep is important for the development of the synapse in the brain (Peirano et al., 2003) and directs energy to allow the release of significant concentrations of growth hormone (Benington and Craig Heller, 1995). For more in depth on hormone regulation and sleep, Hänninen (2007) reviews sleep and rest in young calves. REM sleep in developing neonates is positively correlated with brain size, therefore REM sleep has potential to be involved with brain development and memory (Siegel, 2005). With this understanding, it is important for neonatal animals to achieve good quality sleep.

Limited data is available validating calf sleep. Newborn calves have previously been observed to spend 5.5 hours sleeping, however older calves and adult cows only sleep 3 hours in a 24-h period (Hänninen et al., 2008b). In addition, the same lab group was able to develop non-invasive methods to collect EEG activity measuring NREM and REM sleep in group-housed calves post weaning. It was suggested during 09:00 – 14:30 and 16:30 to 07:00, calves slept for 25% of the observation periods in 50 sleep bouts of 5 minutes. Of the 25% of time sleeping, 45% was observed in REM sleep, and 55% in NREM sleep. In a more simplistic method not requiring electrodes on the calves, Hokkanen et al. (2011) was able to develop a neck-based, wireless accelerometer system for measuring the sleep and lying time of calves. This study was successful in determining total sleep from wake behaviors however, it could not determine the difference in NREM and REM sleep. Further evaluation of this non-invasive, wireless accelerometer is needed to allow for continuous data collection of calves in a production environment to better determine what factors may lead to quality sleep.

Sleep and Social Housing

Social companionship and sleep patterns has barely scraped the surface in farm animals, however there is a decent amount of data from laboratory animals. Rats housed in a group of 4 returned to sleeping behaviors sooner when disrupted from sleep than individually housed rats (Sharp et al., 2002). Beagles housed in pairs were observed to be less vocal and spent more time sleeping than individually housed beagles (Hetts, 1992). In the literature that is available in dairy calves, Babu et al. (2004) used behavioral observation to determine sleep in calves 1 hour before and 1 hour after the delivery of milk. During this observation period, calves housed individually spent more time sleeping than grouped calves, thus suggesting socially housed calves were more active by either eating/drinking at buckets, or in some form of social contact. In Babu et al. (2004) study, there was a limited window of observation, and only behavior was used to determine sleep patterns, which may not be sever an accurate representation of total sleep for a 24 hour period. However, behavioral observation has been validated as a successful measure to determine sleep states in young calves (Hänninen et al., 2008b). As previously mentioned, calves housed with their dam in relation to individual calves, participated in more NREM sleep and had shorter latency to behavioral sleep after ingestion of milk (Hänninen et al., 2008a). In contrast Hänninen et al. (2010) found that there was not a deprivation in REM sleep of calves housed individually in relation to socially housed calves. Thus far, data is inconsistent as to what level of social contact (dam or group housing) can influence sleep quality of dairy calves.

Standing Behaviors

Similar to lying behaviors, standing time has many interpretations. Standing time can be characterized as idle standing (Babu et al., 2004), time eating (Phillips, 2004), response to

hunger (De Paula Vieira et al., 2008), and response to painful procedures, such as castration (White et al., 2008). Time idly standing is not a desirable behavior, due to producers wanting calves to be using their time successfully while standing, such as eating, ruminating, and developing muscle. Dependent on the circumstance, standing behaviors can be driven by positive or negative responses to their environment, and the interpretation must go further than the quantity of standing time reported.

Standing Behaviors of Socially Housed Calves

Standing time can be manipulated by many different environmental and management practices, and warrants careful interpretation (Table 1). For instance, individual calves were observed by Hill et al. (2013) in all seasons and noticed that standing time did not differ among seasons, and on average was 303 min/d. In social housing, dairy calf standing time is variable. Chua et al. (2002) demonstrated that paired calves stood inactive more often than individual calves, while Duve and Jensen (2012) observed paired calves standing in synchrochony for play or feeding. Standing synchrony may be due to partner disruption or social facilitation encouraging the other calf to play or eat. Nevertheless, behaviors completed in synchrony have previously demonstrated animals forming a bond (Gygax et al., 2010). In regards to social housing and the provisions of enrichment, such as ryegrass, group-housed calves resulted in reducing lying time, and increased rumination while standing in relation to individual calves given ryegrass (Phillips, 2004). These data suggests grouped calves were eating more often while standing and using their time standing more efficiently. Additionally, early rumination is beneficial to neonatal calves by accelerating rumen development (Williams et al., 2010).

Play Behaviors of Individual and Social Calves

Increased standing time could be an indicator of calves using their time to eat or play. Locomotor play has been identified by (Jensen et al., 2015) as galloping with changes in direction, bucking, hind-leg-kicking, and body and head rotations. Locomotor behavior has been previously documented as a beneficial tool for how calf welfare is affected by management practices (Krachun et al., 2010). Social play is where the calves can experience the same locomotor play behaviors, but with a social partner. Objective play has been described by Pempek et al. (2016) as calves playing with objects in their pens, such as grain and water buckets.

When observing cattle in a more natural environment, (Reinhardt et al., 1978) observed calves breaking up into groups of 2 or 3 where they grazed and played together, suggesting growing animals in a natural setting value companionship. Furthermore, social play has been suggested a key player for animal development (Spinka et al., 2001).

In conventional rearing of dairy calves, play behaviors may be limited dependent upon age and management practices such as dehorning, milk allocation, space allowance, and group housing. Calves that are reared in small groups by 3 d of age played more often and had more social interactions in relation to calves reared in groups starting at d 7 and 14 of age (Abdelfattah et al., 2018). However, regardless of when calves were housed together in the current study, play behaviors decreased significantly by 6 weeks of age. Other studies have validated that as calves age, play behaviors significantly decrease (Jensen et al., 1998; Jensen and Kyhn, 2000).

In humans, children involved with therapeutic play prior to surgery demonstrated less negative behavior, anxiety, and postpartum pain (T.S.H. and H.L., 2000; William and Violeta,

2008). In animals, castrated piglets played less than control piglets, the pain reduced the want to play (Hay et al., 2003). Disbudding is a common practice in the dairy industry, and it is suggested to cause pain and behavioral stress to calves by reducing play behaviors post surgery (Faulkner and Weary, 2000; Heinrich et al., 2010; Mintline et al., 2013). Three-hours post dehorning, calves that received local anesthetic and NSAID spent more time playing during the observation period in relation to calves that did not receive NSAIDs (Mintline et al., 2013). These calves in the current study were individually housed, therefore it is not known how play behaviors will differ when calves are reared socially and how it may affect painful behaviors.

Space provided in a home pen determines the amount of play behaviors expressed by neonatal calves. When space was reduced for individual and group housed calves, play behaviors were observed less often, regardless of social contact (Jensen et al., 1998). Later in a follow up study from the same lab group, calves given more space participated in more play behaviors in relation to calves in a small pen. Also in the same study, when calves kept in a small pen were exposed to a large arena, locomotor play behaviors significantly increased in relation to the home pen (Jensen and Kyhn, 2000). Calves removed from group housing and confined for 6, 12, 24, and 48 hours performed less locomotive play when introduced to an arena in relation to calves remaining in the group (Sisto and Friend, 2001). These data suggest when calves are given adequate space, calves are more willing to play and may be an indication of better welfare... However, calves housed individually performed more locomotive and objective play behaviors in relation to group housed calves (Jensen et al., 2015; Pempek et al., 2016). Individual calves can participate in more locomotor play, but they lack the ability of social play that may be critical for their long-term development. In a long-term observation, calves performing more

social play early in life are more likely to become dominant cows when entering the lactating herd (Broom and Leaver, 1978).

Play is an indicator of welfare outcomes of calves in various environments. It is important that calves have the appropriate space, given a diet that goes beyond maintenance, and allowed a companion early in life in order for calves to express locomotor or social play that will result in a more successful future.

CALF IMMUNITY

Immunity of Neonatal Calves

The immune system has a broad repertoire of defenses to protect the body from external stimuli (Motivala and Irwin, 2007). However, many factors can alter the immune response. For instance, in laboratory animals, the lack of sleep can lead to negative energy balance, a reduction in health, and later end in fatal bloodstream infections and potentially death (Everson and Toth, 2000). In dairy calves, management practices can influence their immune development. For instance, dystocia reduced the success of passive immunity to calves and increases the risk of morbidity and respiratory disease (Lombard et al., 2007). Surgical procedures, such as dehorning, increases the inflammatory response and increases the risk of pathogen exposure from pulled scabs (Sylvester et al., 1998). Early weaning of young calves reduced neutrophil responses, thus potentially reducing the immune competence (Hulbert et al., 2011b). Although studies have evaluated immunity from different management practices, little is understood on the effects of social housing preweaned dairy calves on immunity.

There is a misconception that calves are born without an immune system. Although calves are born without the presence of antibodies, they still have an innate immunity for

protection. Therefore, instead of neonatal calves lacking an immunity, calves are not immune competent, and rely on maternal antibodies to compensate via colostrum ingestion (Kampen et al., 2006; Hulbert and Moisé, 2016). More in depth of calf immunity can be found in reviews by Barrington and Parish (2001) and Chase et al. (2008). Also, outward health (scours and respiratory diseases) of socially housed calves has been recently reviewed by Costa et al. (2016). However, calf immune competence in relationship to social housing is lacking in previous reviews. In this literature review, efforts will be made to discuss how social housing can affect calves at a cellular level.

Consider Colostrum

Colostrum, or the first milk, is the primary source of nutrients, cytokines, antibodies, and immune cells for the newborn calf. Antibodies from colostrum are present in calves for weeks/months dependent upon the amount, how fast colostrum is fed, and the quality (Chase et al., 2008). Calves that are deprived of colostrum have been observed to have meniscal amounts of immunoglobulins during the first 3 d of life (Clover and Zarkower, 1980). Although colostrum management after collection is vital for the passive transfer and immune development, cow management during the dry period also has a major impact on calf success. A recent study suggests that continuous milking and omitting the dry period of dairy cows resulted in lower plasma natural antibodies in calves for the first two weeks postpartum (Mayasari et al., 2015). Additionally, cows managed during the dry period without the ability to eliminate heat stress, resulted in poor colostrum and poor performing calves (Monteiro et al., 2016). Various forms of management practices can determine the success and longevity of colostrum for the calf. When

measuring immune competence, colostrum quality and quantity must be considered in future studies.

Social Housing and Immunity

Previously reviewed, Bartolomucci (2007) summarized that sex, social dominance, and the interactions with space allowance and resources available all impact the immune function of socially housed rodents. In dairy cattle, very few studies have documented immune changes of calves reared in groups. For instance, Sisto and Friend (2001) evaluated calves at 8 d of age that were isolated for 6, 12, 24, and 48 h. Lymphocyte counts were lower in calves confined for 12 hours, but no trend was established across hours to suggest a consistent effect (Friend et al., 1987). The author addressed the limitation that 48 hours may not have been enough time in confinement to determine a difference in lymphocyte proliferation or apoptosis. A typical measure of acute stress is evaluating total neutrophils and lymphocytes, and is reported as N:L. In dairy cattle, acute stress causes an increase in total neutrophils, and a reduction in total lymphocytes (Friend et al., 1987).

There is a lack of immunological chronic stress (cytokine production and leukocyte numbers) responses evaluated of calves in different housing conditions. In one effort to measure chronic stress in dairy calves, Wilcox et al. (2013) observed calves isolated from their peers for 24 hours and reintroduced to novel calves for 28 hours, experienced adrenal fatigue, suggesting long lasting stress in these animals. In another effort to measure chronic stress, veal calves housed in groups of 8 had elevated expressions of IL-1 and TAC1, and up regulated leukocytes (Abdelfattah et al., 2015). Cough scores were also higher in those kept in groups of 8 in relation to groups of 4 or 2. Therefore, these data suggest that housing of veal calves in larger groups

during the finishing period may lead to greater incidence of respiratory disease, and housing calves in smaller groups can reduce incidences of immune suppression. It is also important to note that these calves in the current study were grouped later in age, and were individually housed for the first 2-3 weeks of life.

In a study that observed calves socialized earlier in life, Jensen and Larsen (2014) observed calves socialized at 1, 2, or 6 weeks of age, did not differ in incidences of scours or antibody development to 5 common pathogens in feces. In addition, age when socially housed did not affect serum antibodies against 3 most common respiratory pathogens. In a recent study Abdelfattah et al. (2018), tried to determine the health and immune differences in calves socialized at different ages. The results of this study indicated that regardless of age at mixing (3, 7, or 14 d), there were no adverse effects on scours, respiratory disease, lymphocyte or neutrophil counts, thus suggesting early social housing does not negatively affect the immune development of calves.

These previous data suggests that emphasis on calf management (cleanliness, milk allowance, space) during the milk feeding state may prevent pathogen loads and respiratory disease in socially housed calves. In order to further support social housing does not negatively affect immunity of preweaned calves, more investigation is needed. Upcoming studies are encouraged to challenge the calf's immunity i.e., with a novel antigen or bacterium, to determine how calves respond in individual and group housing systems.

Pain and Stress

Chronic and acute pain has previously been associated with causing inflammatory responses, i.e. increased secretion of cytokines interleukin (IL)-6 and tumor necrosis factor-alpha

(TNF- α) (Üçeyler et al., 2011). In humans diagnosed with fibromyalgia, increased levels of inflammatory cytokines were related with higher pain severity (Watkins et al., 1995; Menzies et al., 2013). Although a relationship between pain and immunity is clear in humans, however less is known about pain and immune responses in dairy calves. Calves castrated without the present of NSAIDS, had increased circulating leukocytes and haptoglobin, less responsive to *Escherichia coli*, and had less TNF- α responses when challenged with LPS (Ballou et al., 2013). Similarly, castrated bull calves that did not receive pain medication resulted in higher counts of *E. coli* and had higher concentrations of salivary cortisol (González et al., 2010). These data suggest the pain from castration suppressed the immune response of young dairy bulls, and provides a linkage with stress and immune response.

The problem with stress is there are too many interpretations of what it can mean (Bartolomucci, 2007). In dairy cattle, stress can be observed by many different management practices, such as: age of separation from newborn calf and dam (Weary and Chua, 2000), transportation of neonatal calves to calf rearing facility (Hulbert et al., 2011a), and pain from castration or dehorning (Ballou et al., 2013; Molaei et al., 2015). Stress from pain can suppress the immune system by releasing cortisol, which may lead to depression and anxiety (Grace et al., 2014). Stress in utero can also negatively affect dairy calf immunity. For instance, Cows metabolically stressed during late gestation had greater serum concentrations to reactive oxygen and nitrogen species and higher basal inflammation (greater levels of TNF- α) in relation to cows that were not inflicted with metabolic stress (Ling et al., 2018).

Animal Personality, Stress, and Social Interactions

Pain from disbudding and the behaviors, performance, and physiological responses have been previously reviewed via meta-analysis by (Winder et al., 2018). However, little evidence exists on how pain in dairy calves can alter the affective state of calves. In a first attempt to measure pain and cognitive bias in dairy calves, Neave et al. (2013) observed pessimistic negative bias post hot-iron disbudding. Prior to disbudding, calves were trained to approach a screen dependent upon the colors presented. For instance, white meant a reward would be presented if approached (milk from a teat), red meant no reward and a one-minute-time-out if they did approach, or an ambiguous color (mixture of red and white) that had no repercussions if approached. After hot-iron disbudding, calves were given the same approach test. Calves then associated the ambiguous colors as negative for up to 22 hours post dehorning. These data suggest that pain from dehorning caused anxiety in calves and negatively affected their judgement. In agreement with these data, anxiety in other species initiated bias at a near-negative experience as well (Burman et al., 2008; Matheson et al., 2008). To elaborate further, in non-human measures of optimistic or pessimistic experience in animals, they must be given the opportunity to make discriminatory decisions (Harding et al., 2004; Paul et al., 2005). In a recent study with young calves, individual baseline levels of pessimism and optimism were observed. For instance, dairy calves that were pessimistic (hesitation to approach ambiguous places within an experimental pen, and less likely to approach a human) were also more fearful during a novelty test. In addition, rats that carry pessimistic perspectives to stressful stimuli, are more likely to develop depression (Rygula et al., 2013). In agreeance, Human patients diagnosed with depression have previously been linked to greater pro-inflammatory cytokine secretion

(Goldsmith et al., 2016). People with a pessimistic outlook had reduced immune functionality when presented with unfamiliar situations (Segerstrom, 2005). The research in other species brings promise to better understand the pessimistic and optimistic views of farm animals. This understanding will be critical for measuring the affective states of welfare, i.e., calves reared in isolation or in social environments.

The social environment can be dynamic, and depending upon the situation, socializing animals may reduce the welfare and increase stress in laboratory rodents (Bartolomucci, 2007). For instance, mice of both sexes mixed together brought out winners and losers. Dominant male mice were more socially active, but were aggressive towards subordinate pen-mates. In contrast, Morrison and Hill (1967) observed rats with social support visited nutritional resources more often, and tolerated more pain to reach those resources in relation to individual rats. These data suggest that social support and social facilitation reduced the sensitivity and fear to painful stimuli. Though social support during stressful stimuli may benefit other species, it is unclear in dairy calves.

It has also been suggested that calves transitioned from small groups to larger groups reduces stress responses (Bach et al., 2010). In addition, dairy calves housed in isolation altered the hypothalamo-hypophyseal-adrenal axis function, suggesting that deprivation of interaction with conspecifics caused a physiological stress response (Creel and Albright, 1988). Stress in calves is typically measured using vocalizations to novel environments. For instance, when calves were reared in pairs prior to weaning, they participated in less stress vocalizations when introduced to the weaning pen in relation to individual calves (De Paula Vieira et al., 2010). Calves paired together at d 5, were less vocal at weaning than calves housed individually or

paired at 28 d of age, suggesting providing social contact earlier in life has the potential to buffer stress responses during stress-induced situations (Bolt et al., 2017). However, future studies are encouraged to evaluate social housing and its effect on alleviating stress during the milk-feeding stages of dairy calves, such as castration and disbudding.

CONCLUSION

Providing research-based benchmarks for producers of preweaned dairy calves has the potential to encourage producers to adopt social housing calves earlier than at time of weaning. More investigations are necessary to achieve this goal, and behaviors and immune responses to social housing should continue to be investigated. Calves should also be followed later than the weaning stage to determine if it may positively influence their productivity and welfare in the long-term.

CHAPTER II

THE EFFECT OF INDIVIDUAL VERSUS PAIR HOUSING DAIRY

CALVES ON BEHAVIOR AND GROWTH

ABSTRACT

Our objective was to determine the effect of pair versus individual housing of calves on behavior and growth. At 5 ± 1 d relative to birth, calves with successful passive transfer of immunoglobulins from colostrum (STP reading ≥ 5.5 g/dL) were blocked by sex and birth date and assigned to either pair ($n = 14$) or individual ($n = 14$) housing. Calf pairing was implemented by combining two individual pens. One paired calf served as the focal calf and the other imposed treatment. All data were collected from the focal calf in pair housing. Control calves remained individually housed. Milk replacer (protein 26%: fat 20%; 3L) was fed $2 \times$ d and grain and water were provided ad libitum. Accelerometers were affixed on the calf's rear leg on $d 8 \pm 1$, relative to birth, to monitor standing time. Body weight was recorded weekly, at weaning, and 1 week post-weaning. Video observations occurred at early (16 – 18 d of age) and late (42 – 44 d of age) ages for 3 consecutive d. Calves were observed within the pen via 10 min scan samples, and hunger and anticipation behaviors at 1 min scan samples at before the bottle was given, after the bottle was taken away, midday, and midnight observation times. A MIXED procedure (SAS 9.4, Cary, NC) was used to run multiple ANOVAs to evaluate the effects of housing treatments on standing time, bouts, bout duration, and growth. GLIMMIX procedures evaluated behaviors collected by video. Paired calves had greater standing time in relation to individually housed calves. ADG, DM, feed refusals, and growth at weaning to one-week-post weaning did not differ between housing treatments. For the pen utilization observation period, paired calves spent more time awake, and individual calves projected their head out of the pen more frequently. Hunger and anticipation behaviors did not differ between housing treatments. Paired calves increased their standing time, and spent more time awake, thus suggesting paired calves were more active

during the preweaning phase. The increased activity of pair housed calves may suggest improved welfare of preweaned calves.

INTRODUCTION

Rearing calves individually during the milk feeding stage poorly affects calf welfare. Calves raised without contact with conspecifics are more fearful to novel objects and are more aggressive when introduced to new calves in relation to socially reared calves (Jensen et al., 1997; De Paula Vieira et al., 2012). Early social housing of preweaned dairy calves in the U.S. has increased by 9.2% from 2011 to 2014 (USDA 2011; USDA 2014). However, individual housing is still the primary management practice for neonatal calves (69.7%; USDA, 2014). Yet, early social housing of dairy calves encourages social behavior, improves cognitive function, and promotes weight gain (De Paula Vieira et al., 2010; Costa et al., 2015; Meagher et al., 2015).

Socializing young calves promotes social play (Spinka et al., 2001), social bonding, and allogrooming (Wormsbecher et al., 2017). During the milk feeding stage, individual calves spend more time with their head out of the pen, suggesting the need for companionship (Chua et al., 2002). Calves housed together by 4 d of age, were more successful at reversal learning to color changes and milk rewards, whereas individual calves were less successful. These data suggest socially housed calves have greater cognitive abilities (Meagher et al., 2015). In addition, early grouped calves in relation to individual calves were less reactive to novelty in the environment at later observations (De Paula Vieira et al., 2012). At weaning, paired calves were more successful at using an automated calf starter system and maintained body weight, whereas individual calves were less successful and lost weight (De Paula Vieira et al., 2010). Finally, Raussi et al. (2010) observed early grouping of calves allows for bonds to last up to 1.5 years of age. Social housing calves during the preweaning stage results in more successful calves, however behaviors and performance rates that lead to this success are not clear.

Although calves may benefit from social housing earlier in life, behaviors seem to be inconsistent or unknown between socially and individually housed calves. For instance, play increased when calves in small groups had more space within the pen (Jensen and Kyhn, 2000). In addition, Jensen et al. (2015) observed paired calves to play more frequently when milk allowance increased and space allowance was the same. However, Pempek et al. (2016) observed paired and individual calves not differing in locomotor play behavior. Through these data, it is unclear if the presence of a social partner, space, or milk allowance is more important for calves to express play behaviors. In addition, calf resting posture has been observed in grouped and individual calves, however calves were evaluated on how posture varied between indoor and outdoor systems, and it is unclear how the sole effect of social housing can influence lying postures (Hänninen et al., 2010). Previous data is either inconsistent or unclear on how social housing calves during the preweaning state can alter specific behaviors within a given environment that may be beneficial for calf development.

Growth is a critical biological welfare component in neonatal calves and the initiative to ingest solids sooner may be dependent upon a social partner. However, starter intake of socially reared dairy calves is inconsistent thus far. Chua et al. (2002) and Abdelfattah et al. (2018) observed no differences in starter intake of individual or grouped calves during the milk feeding phase. In contrast, Babu et al. (2004) observed grouped calves spending more time eating solid feeds. Furthermore, calves socially housed during the milk feeding stage had greater feed intake and weight gains prior to and after weaning (Bernal-Rigoli et al., 2012; Costa et al., 2015). Socially housed calves ingesting concentrate sooner has previously been linked to social facilitation (Miller-Cushon and DeVries, 2016). However, these differences in growth may also

be dependent upon management practices (milk allowance and weaning methods) between studies. Regardless, these data are inconsistent and need to be explored more.

There is evidence that dairy calves socialized earlier in life are more successful, however the specific behaviors that promote this positive change have yet to be established. The primary objective of this study was to determine how behavior of calves reared individually or paired differ, and determine how behaviors adapt as calves age. The secondary objective of this study was to evaluate calf performance changes between individual and pair housed calves.

MATERIALS AND METHODS

Animals, Management, and Housing

Holstein calves ($n = 55$) born between December 2016 and May 2017 were enrolled in the current study. Birth weights of calves did not differ between individual (38.4 ± 1.61 kg) and pair (39.6 ± 1.61 kg) housed calves ($P = 0.56$). All calves received on average 1.9 ± 0.5 L of frozen maternal colostrum by the first 3.1 ± 1.4 h of birth. The second feeding of 2.0 ± 0.5 L of colostrum was given by 8 ± 1.7 h after birth. All colostrum was tested via colostrometer (Coburn Orange Rubber Armor Jacket Refractometer, Whitewater, WI) and colostrum with > 50 mg/mL of Ig was fed to the calves. Calves received 3 L (0.34 kg) of milk replacer (AG Central Calf 26-20 BOV SC ClariFly[®], Laverne TN) twice-daily at 0530 and 1630 h through a teat and bottle (Peach Teat[®], New Zealand). The bottles were left with the calves 20 minutes after milk delivery to prevent cross suckling (de Passillé, 2001). Concentrate and water was available to calves ad libitum (18% CP; Co-op Calf Primer/TCR I-BOV, Laverne TN). Calves were weaned on 60 ± 5 d of age.

All calves were housed individually for the first 5 ± 3 d after birth to ensure positive transfer of antibodies and calves could nurse from a bottle and teat independently. Calves were housed in calf pens (Drop-in-Go Indoor Calf Pen System, Agri-plastics, Cortland, NY United States). Graveled pens were covered with deep-bedded straw, and new bedding was added twice daily. A metal roof with open sides for natural airflow covered calf pens.

All procedures used for this project were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee, Knoxville.

Treatment

A blood sample was collected on d 3 ± 2 of age to evaluate serum total protein (STP) in order to determine successful passive transfer of antibodies from colostrum. Samples were collected in a 5-mL Vacutainer serum collection tube (BD Vacutainer Venous Blood Collection Tubes, Vacutainer Plus Plastic Serum Tubes, Silicone-Coated with Hemogard Closure, Fisher Scientific, Pittsburgh, PA) from 20G needle and vacutainer holder. Serum samples were separated by centrifuge following the same protocol as (Pempek et al., 2016). Only calves with a $STP \geq 5.5$ g/dL determined with a refractometer (Global Industrial MASTER-SUR/Na refractometer, Port Washington, NY) were enrolled.

After d 5 and confirmation that calves had a successful passive transfer of antibodies, calves were allocated to 1 of 2 housing treatments: individual ($n = 14$) or paired ($n = 28$). Pair housing was implemented by removing the middle divider of two pens to double the amount of space (individual housing dimensions: $1.9\text{m} \times 1.1\text{m} \times 1.9\text{m}$; paired housing dimensions: $3.8\text{m} \times 2.2\text{m} \times 3.8\text{m}$). Calves in both housing treatments had the same space/pen.

Video Observation

Pen Utilization. Two cameras (1.0-Megapixel Outdoor IP67 Bullet Cameras. Amcrest, Houston, Texas) were mounted on a wooden 2 × 4 that went across the trusses of the metal roof above the calf pens. Cameras had IR-LED lights for night viewing. Pen utilization of calves were recorded by 10 minute scan sampling to observe behaviors (Table 2) for 3 consecutive d beginning at 16 (16-18 d; early age) and 42 (42-44 d; late age) d of age. Observation began at a given start time, and ten-minutes thereafter served as the 10-minute scan sampling for behaviors to be recorded. Behaviors were observed for 3 consecutive days. These behaviors were dichotomously recorded where 0 indicated the behavior was not taken place at observational time point, and 1 indicated the activity was observed.

Anticipation Behaviors. Anticipation behaviors were recorded at 1-min scan sampling at AM feeding, PM feeding, mid-day (**MD**; 10:30 – 11:30 ± 1 h), and mid-night (Table 2; **MN**; 21:00 – 22:00 ± 1 h; Pempek et al., 2016) . Hunger and anticipation behaviors were recorded similarly to those for pen utilization scan sampling. Anticipation behaviors were categorized as PRE (before bottle given), and POST (after bottle removed) for the AM and PM feeding times. Anticipation observations began 30 minutes prior to milk feeding and 30 minutes after the bottle was removed. Hunger behaviors were evaluated for 1 hour at MD and MN. Regardless of observation (pen utilization or anticipation/hunger behaviors), if a behavior could not be recognized by scan sampling, a 5-second epoch from the stop time was used to define behaviors. Three observers collected behavioral data with an inter-observer reliability of 93 % (± 2.64 SD).

Standing Behaviors

Standing time, bout, and bout duration of calves were recorded using Hobo Pendant G Acceleration electronic data loggers (Onset Computer Corporation, Bourne, MA). Hobo accelerometers were recently used and validated as an accurate way to measure standing and lying activity of dairy calves (Bonk et al., 2013). Every 7 d, accelerometers were removed, downloaded, and replaced on the opposite leg to prevent lesions and loss of data (AWP, 2013).

Performance

At birth, the initial weight of each calf was collected and every Monday until weaning (60 ± 5 d). A final weight was collected 1 week post weaning 67 ± 5 d. Feed refusals were measured for 3 consecutive d of each week by weighing the feed buckets provided to calves in both housing treatments. Feed refusals for paired calves were averaged together (Pempek et al., 2016). Feed samples were also collected to evaluate DM of starter grain for calves in both housing treatments. Fresh water was provided to the calves daily, however water intake was not measured.

Statistical Analyses

Separate mixed model analyses of variance (ANOVA) were performed using PROC MIXED in SAS v9.4 (SAS Institute Inc., Cary, NC) for standing behaviors and performance. Normality was assessed using PROC UNIVARIATE for all dependent variables. The response variables of interest included standing time, standing bouts, standing bout duration, ADG, feed refusal, and DM. The fixed effects of housing treatment, day, and week were included in the analyses, as well as the interactions between these variables. Finally, the correlation procedure

(PROC CORR) was used to determine the relationship between weights at weaning and weights-one-week-post weaning.

Additionally, to determine if there were housing treatment differences in the proportion of time calves spent expressing various feeding or pen utilization behaviors in video data, multivariable, multilevel logistic regression was performed using the GLIMMIX procedure. Behavior events were recorded in a binary fashion, and within a sampling period, binary events were summed to calculate a total number of observed time points at which a calf was expressing behavior. The total number of trials were equivalent to the total number of observational time points for a particular calf. A binomial distribution and logit link were specified and each response was modeled as the number of events over the total number of trials. The fixed effects of housing treatment, age, and feeding times (PRE, POST, MD, and MN) and their interactions, as well as the random effects of Calf ID and repeated measures over time were included in all models. There were behaviors that could only be expressed by calves in paired housing, and these were analyzed to test if there were differences in the probability for calves to exhibit these behaviors due to age (early vs. late). The LS means statement and the ILINK option was used to obtain the mean percentage in which calves were observed expressing behaviors in a specific observational period. Statistical significance for all analyses was determined at $P \leq 0.05$.

RESULTS

Descriptive Statistics

Multiple behaviors in the current study were observed in calves housed individually and in pairs. However, not all of the behaviors occurred frequently enough for statistical analysis.

Behaviors that occurred < 1% of time were unable to converge for further analysis, however these behaviors are represented in a percentage of time observed when necessary in the discussion (idle standing, behaviors classified as other and unknown, ingesting water, locomotor play, social play, together calf proximity, social facilitation, and short resting posture). Behaviors that successfully converged were used for analysis to determine difference between housing treatments or age.

Pen Utilization

Calves in both housing treatments did not differ in posture ($P > 0.43$), play ($P = 0.63$), feeding ($P > 0.11$), oral ($P > 0.15$), and sleep ($P = 0.58$) behaviors for the pen utilization observation period (Table 3). However, paired calves were observed to spend more time in awake behaviors ($P = 0.03$), and individual calves were observed to project their head out of the pen more frequently than paired calves ($P = 0.02$; Table 3). In addition, a housing treatment and age interaction observed paired calves at early age observation participating in less self-grooming behaviors ($P = 0.02$; individual and early = 4.41, individual and late = 4.47, paired and late = 5.28 ± 0.50 vs. paired and early $2.77 \pm 0.43\%$ of time observed).

Paired Calf Behaviors Only

Pen Utilization. Regardless of age, paired calves were not observed to differ in time spent alone ($P = 0.80$; early = 0.71 vs. late = $0.74 \pm 0.08\%$ of time observed), together and touching ($P = 0.22$; early = 11.02 ± 1.42 vs. late = $13.74 \pm 1.57\%$ of time observed), allogrooming ($P = 0.30$; early = 0.02 vs. late = $0.01 \pm 0.01\%$ of time observed), or cross suckling ($P = 0.32$; early = 0.02 vs. late = $0.03 \pm 0.01\%$ of time observed).

Anticipation Behaviors. Cross suckling of paired calves did not differ at specific feeding times ($P = 0.32$; PRE = 0.03, POST = 0.03, MD = 0.02, MN = $0.01 \pm 0.01\%$ of time observed). However, an age and observation interaction was observed for cross suckling (Figure 1; $P = 0.03$).

Anticipation Behaviors

Calves in both housing treatments did not differ in anticipation and hunger behaviors (Table 4), however anticipation behaviors in all calves were dependent upon time observation (Table 5). These behaviors included lying postures ($P = 0.24$), ingesting grain ($P = 0.75$), licking the pen ($P = 0.13$), eating straw ($P = 0.66$), self-grooming ($P = 0.17$), and head out of the pen (Table 4; $P = 0.72$). Additionally, housing treatment and age interaction found individual calves ingesting grain more often at early age observation ($P = 0.02$; individual and early = 4.44, paired and early = 2.67, paired and late = 3.04 ± 1.3 vs. individual and late = $1.30 \pm 0.62\%$ of time observed). Another interaction observed individual calves at early age licking the pen more often than any other age group or housing treatment ($P = 0.05$; individual and early = 8.78 ± 2.57 vs. individual and late = 4.06, paired and early = 2.77, paired and late = $3.16 \pm 1.2\%$ of time observed).

Standing Behaviors

Paired calves had greater standing time than individual calves (Figure 2; $P = 0.004$; paired = 6.10; individual = 4.69 ± 0.32 h/d). Standing bouts did not differ in relation to housing treatment (Figure 2; $P = 0.12$). However, a tendency for paired calves to have greater mean standing bout durations was observed (Figure 2; $P = 0.08$).

Performance

Calves in both housing treatments did not differ in performance (Figure 3). The ADG ($P = 0.35$; individual = 0.50 vs. paired = 0.53 ± 0.03 kg), and feed refusals ($P = 0.43$; paired = 0.63 vs individual = 0.56 ± 0.05 kg) did not differ between housing treatments. The change in growth at weaning to one-week-post weaning did not differ between housing treatments ($P = 0.88$; paired = 3.08; individual = 3.30 ± 0.99 kg).

DISCUSSION

Behaviors of calves housed individually or in small groups have previously been observed around milk feeding times or at 24-h observations. This study was unique in that individual and pair housed calves were observed using both methods at 2 different ages. In addition, evaluating standing behavior using an accelerometer in calves housed individually and in a pair has yet to be conducted. Paired calves in the current study were recorded to have greater standing time and spent more time in awake behaviors. Individual calves were observed to project their head out of the pen more often than paired calves. A housing treatment and age interaction observed paired calves at early age participating in less self-grooming behaviors and more cross-suckling behaviors at PRE and POST observation times. Individual calves at early age ingested grain and licked the pen more frequently than any other age and housing treatment. The increased standing time and time spent awake in paired calves may suggest these calves were more active than individual calves. Individual calves ingesting grain, licking the pen more frequently at early age, and sticking head out of the pen more often may suggest these calves were more curious of their environment, and looking for companionship.

Paired calves in the current study were observed to have greater standing time than individual calves. Evaluating the standing time of young calves warrants careful interpretation. For instance, White et al. (2008) observed calves to stand more often after castration, suggesting standing longer periods is a pain response. Bull calves in the current study were not castrated, therefore the greater time paired calves were observed to be standing might be suggested as a positive outcome. Increased standing time may be suggested as a positive outcome due to the lack of differences in standing bouts between individual and pair housed calves. Greater bouts in pair housed calves would have suggested that the social partner disrupted the rest another calf. The non-focal paired calf did not wear an accelerometer, and may have limited our ability to determine if calves were disrupting the non-focal calf or vice versa. It is important to note all calves in the current study spent more than 18 h/d in lying time. The amount of time lying in the current study is in agreement with previous work that has observed conventional preweaned calves on clean, dry bedding lying more than 17 h/d (Camiloti et al., 2012; Bonk et al., 2013). The ability to meet lying and resting requirements are important for the growth and development of young calves, and has negative outcomes if restricted (Mogensen et al., 1997; Hänninen et al., 2005).

In the current study, paired calves had greater standing time, which is in agreement with previous work (Chua et al., 2002; Phillips, 2004). The commonality between the current and previous studies may be from a greater desire for calves to move around, even though space/calf was similar regardless of housing treatment. Phillips (2004) observed grouped calves having access to various forms of forages to have greater standing time than individual calves with the same forage types. Calves in the current study were housed on deep-bedded straw where soiled

straw was removed and fresh straw was added twice daily, and may have stimulated calves to stand and explore the straw bedding, however no differences of eating straw between housing treatments were observed in the current study. Time spent sniffing the straw was not documented, and may have limited our interpretation of calves' curiosity of the straw. Other factors other than enrichment may also influence greater standing time in dairy calves, such as play and social facilitation (one calf mimicking the behavior of another calf). For instance, paired calves in the current study were observed to participate in 0.18% of time observed in social play and 0.02% time observed in social facilitation. Duve and Jensen (2012) observed standing time of paired and individual calves to not differ, however paired calves stood in synchrochony. From previous data, social housing calves at similar ages influences synchrony behaviors, which may be initiated by social gestures and social facilitation. Standing synchrony was not measured in the current study, and may have impacted our results. Furthermore, future studies should observe more explorative behaviors of individual and pair housed calves, such as sniffing the pen and air, nesting, and looking over the pen to determine if these may influence greater standing time.

The increased standing time may be related to observations of paired calves spending more time awake. During the pen utilization observation, paired calves spent more time awake (76.2%) in relation to individual calves (70.3%). Calves were considered to be awake when their heads were up and moving when laying down or standing. Behaviors in neonatal calves are sufficient to determine if calves are expressing sleep or wake behaviors; however, this is not the case in adult cattle (Hänninen et al., 2008b; Ternman et al., 2012). In contrast to the current study, pair-housed beagles, were observed to spend more time in asleep behaviors (Hetts, 1992). In addition, when individual and group housed rats were disrupted from sleep, grouped rats were

observed to have a shorter latency to fall back asleep. Latency to be in sleep states were not observed in the current study and may have limited our interpretation of sleep quality between housing treatments. However, calves housed with the dam spent more time awake during the colostrum feeding stage in relation to calves fed colostrum through a teat, and tended to have a shorter latency to express NREM behaviors (Hänninen et al., 2008a). In agreement with the current study, Babu et al. (2004) observed grouped calves spending more time in awake behaviors in relation to individual calves. However, scholars from the same lab group did not observe a difference in REM sleep between individual and group housed calves when exposed to indoor or outdoor housing (Hänninen et al., 2010). Previous data along the current study, suggests social housing of animals may result in animals spending more time awake or having better quality rest. Furthermore, the time spent awake may be from social bonding or exploring the pen, however the quality of sleep of socially housed calves during the preweaning phase still remains unclear. More intense methods are necessary to assess sleep quality in calves. For instance, surface electrodes on recently weaned calves has successfully differentiated non-rapid eye movement (NREM) and rapid eye movement (REM) sleep (Hänninen et al., 2008b). Surface electrodes were used in the current study, however these data are not shown. Further evaluation of sleep states in calves in the current study will provide insight of sleep states of individual and paired calves at early and late age observations.

Cross suckling in socially housed dairy calves has been reported in all grouped preweaned dairy calf literature. Cross suckling, however, can be mitigated by milk flow and portion size (Roth et al., 2008; Nielsen et al., 2018), introducing forage enrichments (Phillips, 2004), or an artificial dry nipple (Jung and Lidfors, 2001). Although bottles were left in the pen

with calves 20 minutes after ingestion of milk, cross suckling was observed 2.3% of the total proportion of time observed. The observations of cross suckling in the current study was greater than $\geq 0.15\%$ (Chua et al., 2002; Wormsbecher et al., 2017), roughly the same as $< 3.4\%$ (Lidfors, 1993; Nielsen et al., 2008) and less than 13.5% (Loberg and Lidfors, 2001; Pempek et al., 2016) previous work. These data demonstrate that cross suckling is controllable, but cannot be eliminated, and may be dependent on age. At early age, pair housed calves performed most cross-suckling behaviors at PRE and POST observation periods, however the percentage of cross-suckling behaviors decreased by late age observation. The current data are in agreement with previous work in which calves decrease the amount of cross-suckling before and after milk replacer as they get older (Nielsen et al., 2008). Therefore, these data suggest that social calves are more anxious for the bottle earlier in age, and are able to learn habits later in life. In addition, calves may begin to fixate oral behaviors elsewhere within the pen.

The increase in cross suckling of paired calves at early age observation may be from paired calves participating in self-grooming less often at early age observation. Paired calves at early age observation were self-grooming less often than individual calves at both ages, and less than late observation of paired calves. These data are in somewhat agreement with Pempek et al. (2016), where individual calves spent more time self-grooming, however individual calves in the current study did not differ in self-grooming from paired calves at late age observation. Furthermore, paired calves may have groomed themselves less due to only being housed for roughly 10 d at this point and were more curious of penmates.

Self-grooming in individual calves can be thought of as a boredom response. In addition, individual calves in social deprivation have previously been observed to seek companionship of

other calves by projecting their head out of the pen, which may also be a boredom response (Chua et al., 2002). Likewise, individual calves in the current study were observed to project their head out of the pen 2.8% of time observed and paired calves projected head out of the pen .37% of time observed. Previously, calves stuck their head out of the pen more frequently when separated from cows 14 d after birth, thus suggesting head out of the pen is a response to social deprivation (Flower and Weary, 2001). Individual and pair housed calves were both observed to project their head out of the pen in relation to bottle delivery, however no housing treatment differences were observed during the feeding period observations. Therefore, individual calves projecting their head out of the pen in the current and Chua et al. (2002) study may have been an attempt to search for companionship of calves in adjacent pens.

Hunger and anticipation behaviors did not differ between individual and pair housed calves. However, in a housing treatment and age interaction, individual calves at early age were observed to ingest grain and lick the pen more often than other housing treatments and age groups. The observation of individual calves ingesting grain at early age observation was unexpected, due to other studies detecting the opposite (Costa et al., 2015; Pempek et al., 2016; Wormsbecher et al., 2017). However, ungulates have been observed to increase curiosity and ingest grain at an early age (Key and Maciver, 1980; Thorhallsdottir et al., 1990). Therefore, video data collection in the current study could have captured individual calves becoming more curious to calf starter at an early age. In addition, individual calves at early age were observed to lick the pen more often during feeding time observations. These data may be related to individual calves ingesting grain more often at early age observation, expressing curious behaviors within

the pen, not fully understanding the habit of bottle feeding times, and potential boredom from the lack of stimulation in the environment.

Calves in both housing treatments did not differ in ADG, DM, feed refusal, and growth rates from weaning to one-week-post-weaning. Abdelfattah, et al. (2018) recently observed similar results. Additionally, Jensen et al. (2015) agreed regardless of social housing, calves did not differ in growth. Chua et al. (2002) observed paired and individual calves having similar ADG, however at time of weaning, individual calves were only gaining one half kg/d, whereas pair housed calves were gaining 1 kg/d. Similarly, De Paula Vieira et al. (2010) observed paired calves to have similar weight gain during the milk feeding phase, but individual calves lost weight at the beginning of the weaning period due to the latency to comprehend an automated grain feeding system. In the current study, calves at weaning were fed ad libitum grain in an easy-to-use trough, which may have allowed all calves to easily consume grain. In addition, calves were only observed 1 week after weaning, and may not have been enough time to see a difference in coping abilities from weight gain between housing treatments. The difference in performance is highly dependent on management practices (milk allowance, number of animals, duration of study, and technology), and each can alter the growth outcomes of socially housed calves.

CONCLUSIONS

In conclusion, paired calves in the current study were recorded to have increased standing time and spent more time awake in relation to individually housed calves. Individual calves were observed to project their head out of the pen more often than paired calves. A housing treatment and age interaction observed paired calves at early age participating in less self-grooming

behaviors. A time and age interaction observed paired calves cross suckling more at PRE and POST observation times in relation to other feeding time and later age observations. Individual calves at early age ingested grain and licked the pen more frequently than any other age and housing treatment. These data suggest that paired calves are more active during the preweaning phase, and may be a positive outcome of early socialization.

CHAPTER III

THE EFFECT OF INDIVIDUAL VERSUS PAIR HOUSING DAIRY CALVES ON IMMUNE COMPETENCE AND PAIN SENSITIVITY

ABSTRACT

Our objective was to determine the effects of pair vs individual housing on immune competence and pain sensitivity in Holstein calves. Calves with successful passive transfer of immunoglobulins (STP > 5.5 g/dL) were enrolled into pair (n = 28) or individual (n = 14) housing on 5 ± 1 d postpartum. Regardless of treatment, 2.1 m² of housing space/calf was available. In the paired treatment, a focal calf was used for all data collection, while the non-focal calf was used as a means to implement the housing treatment. Milk replacer (protein 26 %; fat 20 %; 3L) was fed twice daily, and grain and water were provided ad libitum. On d 7 ± 3 postpartum, calves were injected with 1 mL of keyhole limpet hemocyanin (KLH), Quil-A adjuvant, and nonpyrogenic saline. A secondary injection was given on d 21 ± 3 d including KLH, Quil-A, and heat-killed *Candida albicans* (CA) in nonpyrogenic saline. Serum samples were collected on 3, 14, 28, and 35 d of age. IgG and IgM concentrations from each collection day were evaluated by ELISAs. On d 28 ± 3 d, calves were given with intradermal injections of CA (2×10^6 cells) or saline in the neck to evaluate delayed type hypersensitivity. Injection sites were measured in centimeters with calipers at 0, 6, 24, and 48 h post-injections. A stimulation index was calculated by mean CA response over saline response. Calves were disbudded at 35 ± 10 d of age. Pain sensitivity to disbudding was measured at -1, 3, 27, 51, and 75 h. Separate linear MIXED models (SAS 9.4, Cary, NC) were used to evaluate the effects of housing treatments on IgG, IgM, CA reactions, and pain sensitivity. IgG and IgM ELISA units to KLH did not differ between housing treatments. IgG ELISA units at d 14 were lower than d 3, 28, and 35 in all calves. IgM ELISA units were highest at d 3, but continued to drop thereafter. The stimulation index for delayed hypersensitivity to CA did not differ between housing treatments.

However, injection site reactions increased over time, and peaked at 24 and 48 h post CA injections. Pain sensitivity did not differ between housing treatments, however calves increased sensitivity at 27, 51, and 75 h, relative to disbudding. Pair housing dairy calves during the preweaned period did not negatively affect the immune development of dairy calves or pain from disbudding. This suggests social housing calves early in life does not result in immune dysregulation, or increases pain sensitivity after disbudding.

INTRODUCTION

Social isolation of neonatal calves has negative welfare outcomes. Calves housed alone vocalize more at weaning (Bolt et al., 2017), and ingest less concentrate during the weaning phase (Bernal-Rigoli et al., 2012). Furthermore, housing calves individually has a harmful effect on public perception (Ventura et al., 2016). While group-housing calves has increased 9.2% since the 2011 NAHMS census, approximately 70% of calves in the U.S. are reared individually during the preweaning stage (USDA, 2014). Welfare issues can be mitigated by rearing calves in a social environment during the preweaning phase. A potential benefit from social housing that has not been fully explored, is improved immune competence of calves socialized during the preweaning phase. The hygiene hypothesis indicates humans and laboratory animals exposed to antigens at an early age decreases the prevalence of an overactive immune system (Strachan, 1989a; Bach, 2002). Therefore, it may be hypothesized the same affect can occur in preweaned dairy calves. However, it is important to note that cattle, unlike humans and mice, have a cotyledonary placental type and do not transfer antibodies in utero. The maternal antibodies ingested via colostrum can play a major role in calf immune development. Nonetheless, previous literature demonstrating calves with successful transfer of colostral antibodies minimizes the

incidences of outward health diseases when introduced to one another earlier in life (Costa et al., 2016). In addition to the protective start of colostral antibodies, exposure to antigens in the environment sooner from social housing may allow for a more robust immune system for preweaned calves.

The hygiene hypothesis theory argues restriction of antigen exposure in the environment can negatively affect immune function (Okada et al., 2010). Occurrences of antigen restriction can be as simple as social deprivation. For example, humans raised with siblings or exposed to other children as infants, tend to have a more robust immunity (Strachan, 1989b; Ponsonby et al., 2005; Cardwell et al., 2008). Immunological benefits from social housing has also been observed in other species. Laboratory rodents raised in groups have greater survival rates (Bartolomucci, 2007), primates had a more balanced helper T cell to suppressor T cell ratio (Lewis et al., 2000), and grouped piglets had successful lymphocyte function in relation to individual piglets (Kanitz et al., 2003). These data suggest through greater antigen exposure and social contact early in life, a more appropriate balance between effector and immune regulator cells may result.

Although immunity may benefit from a social environment, the effects of early socialization of calves on immunity remain inconsistent. Sisto and Friend (2001) observed 8-day-old calves isolated for 6, 12, 24, and 48 h. Calves in confinement for 12 h had lower blood lymphocyte counts, but no trend was established across all hours to suggest a consistent effect at which level of confinement was most detrimental to calves. Jensen and Larsen (2014) observed individual and socially housed calves for 6 w and detected no differences in scours or antibody development to common pathogens among neonatal calves. In contrast, Abdelfattah et al. (2015) observed that calves in groups of eight had elevated expression of pro-inflammatory cytokines

(IL-1 and TAC1) and up-regulated leukocytes in relation to calves in groups of 2 or 4. However, in a recent study from the same lab group, Abdelfattah et al. (2018), observed that regardless of mixing age of neonatal calves housed in groups of 3, no adverse effects of immunity (lymphocyte and neutrophil counts) occurred. Across previous literature, it may be suggested group size is the contributing factor to neonatal calf immune dysregulation, and necessarily age of contact. Because data-evaluating immunity of socially housed calves remains unreliable, further investigation of immune function and social housing is necessary.

Immunity imbalance, such as the release of pro-inflammatory cytokines, can cause immune dysregulation and changes in behavior. For instance, the initiation of inflammatory cytokines release has a positive relationship with pain sensitivity (Watkins et al., 1995; Faulkner and Weary, 2000; Menzies et al., 2013). In dairy calves, non-steroidal anti-inflammatory drugs have previously been validated to reduce pain associated behaviors to disbudding (Winder et al., 2018). However, the sole ability for social housing calves to reduce pain sensitivity remains unclear. In laboratory rats, socially housed rats were less responsive to pain stimuli on the tail in relation to individual rats (Jean-Pierre Coudereau et al., 1997). Human women who were considered to have a strong social support system at time of diagnosis, were less sensitive to pain post-cancer treatment and expressed fewer depressive symptoms in relation to patients considered to have weak social support systems (Hughes et al., 2014). The positive outcomes of social support on pain sensitivity may also be observed in calves during disbudding.

Humoral or cell mediated immune responses have been separately evaluated in individual and socially housed calves; however, utilizing both branches of the acquired immunity to assess immune competence of dairy calves during the preweaning state has yet to be understood. In

addition, a potential relationship may exist between social housing and pain sensitivity of young dairy calves after disbudding. Therefore, our primary objective was to evaluate both branches of the acquired immunity (humoral and cell mediated) on individual and pair housed calves to novel antigens keyhole limpet hemocyanin (KLH) and heat killed *Candida albicans* (CA). Our secondary objective was to measure pain sensitivity after hot iron disbudding in calves of both housing treatments.

MATERIALS AND METHODS

Animals, Management, and Housing

Holstein calves (n = 55) born between December 2016 and May 2017 were enrolled in the current study. Birth weights of calves did not differ between individual (38.4 ± 1.61 kg) and pair (39.6 ± 1.61 kg) housed calves ($P = 0.56$). All calves received on average 1.9 ± 0.5 L of colostrum by the first 3.1 ± 1.4 h of birth. The second feeding of 2.0 ± 0.5 L of was given by 8 ± 1.7 h after birth. All colostrum was tested via colostrometer (Coburn Orange Rubber Armor Jacket f Refractometer, Whitewater, WI) and colostrum with > 50 mg/mL of Ig was fed to the calves. Calves received 3 L (0.34 kg) of milk replacer (AG Central Calf 26-20 BOV SC ClariFly[®], Laverne TN) twice daily beginning at 0530 and 1630 h through a teat and bottle (Peach Teat[®], New Zealand). The bottles were left with the calves 20 minutes after milk delivery to prevent cross suckling (de Passillé, 2001). Concentrate and water was available to calves ad libitum (18% CP; Co-op Calf Primer/TCR I-BOV, Laverne TN). Calves were weaned on 60 d (± 5 d) of age.

All procedures used for this project were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee, Knoxville.

All calves were individually housed in deep straw bedded calf pens (Drop-in-Go Indoor Calf Pen System, Agri-plastics, Cortland, NY United States) for the first 5 ± 3 d after birth to ensure positive transfer of antibodies, and calves could nurse from a bottle and teat independently. A metal roof with open sides for natural airflow covered calf pens.

Treatment

A blood sample was collected on d 3 ± 2 to evaluate serum total protein (**STP**) to determine absorption of immunoglobulins from colostrum. Samples were collected in a 5-mL Vacutainer serum collection tube (BD Vacutainer Venous Blood Collection Tubes; Vacutainer Plus Plastic Serum Tubes, Silicone-Coated, with Hemogard Closure, Fisher Scientific, Pittsburgh, PA) from a 20G needles and vacutainer holder. Serum samples were separated by centrifuge following the same protocol as Pempek et al. (2016). Only calves with ≥ 5.5 g / dL determined with a refractometer (Global Industrial MASTER-SUR/Na refractometer, Port Washington, NY) were enrolled.

After d 5 and confirmation that calves had a successful passive transfer, calves were allocated to 1 of 2 housing treatments: individual ($n = 14$) or paired ($n = 28$). Pair housing was implemented by removing the middle divider of two pens to double the amount of space (individual housing dimensions: $1.9\text{m} \times 1.1\text{m} \times 1.9\text{m}$; paired housing dimensions: $3.8\text{m} \times 2.2\text{m} \times 3.8\text{m}$). Calves in both housing treatments had the same space/pen.

Immune Measures

Humoral Immunity. Humoral immunity was evaluated with keyhole limpet hemocyanin (KLH) to assess antibody production (Table 6). On d 7 ± 3 , calves were given a 1 mL primary IM injection in the neck containing KLH (Merck KGaA, Darmstadt, Germany; 0.1 mg), and

Quil-A (InvivoGen, San Diego, CA; 0.5mg) in pyrogen-free saline (Enzo Life Science, Inc. Farmingdale, NY). On 21 ± 3 d, calves received a secondary injection of KLH (0.1mg), Quil-A (0.5mg), and CA (Strain 5314, Todd Reynolds, University of Tennessee, Knoxville) in pyrogen-free saline. Serum was collected using the same method previously described for STP. Serum samples were placed in cryo-vials and stored at -80°C until evaluation of ELISA units (IgG and IgM) specific to KLH. Plates (Costar high binding 3669, Cornington NY) were coated with 100 μL /well of KLH (1 $\mu\text{g}/\text{mL}$) in carbonate coating buffer and were incubated at 4°C overnight. Plates were washed 4 times with $10 \times$ phosphate buffer-Tween (PBT; 0.05% Tween). Plates were blocked with PBT plus 2.5% goat serum (Jackson ImmunoResearch. 101-035-165. West Grove, PA) at 200 μL /well for 30 min at room temperature (RT). Washing followed the same as before. Calf serum sample with the highest ELISA unit to KLH was chosen as the positive control and set the standard curve. The positive control serum was serially diluted with PBT + 2.5% goat serum on the plate (100 μL /well) from 1/500 to 1/32000 in duplicate. The negative control, [Fetal Bovine Serum (FBS)] (Atlanta biologicals. cat. S11550, lot. A1061. Flowery Branch, GA) was diluted 1/1000 in PBT + 2.5% goat serum and plated in duplicate at 100 μL /well. The positive and negative control samples helped set the standard curve for all calf antibody detections to fall within. Calf sera samples were diluted to 1/1000 in which the antibody detections were able to fall within 2/500 and 1/1000, and the concentration levels at that dilution were established as the ELISA unit. Each sera sample from blood collection days was diluted 1/1000 with PBT + 2.5% goat serum (Gibco. ThermoFisher. Waltham, MA) and plated in duplicate at 100 μL /well. The plates then were incubated at RT for 1 h. Plates were washed as before after incubation. Secondary goat anti-bovine antibody diluted 1/2500 with PBT was added

at 100 μ L/well and incubated for 1 h at RT. Plates were washed for the last time. Substrate (BD OptEIA TMB substrate reagent, San Diego, CA) added at 100 μ L/well and incubated for 15 min at RT. Plates were then read at 630nm (Biotec ELx808 Microplate reader, Bridport, UK).

Cell Mediated Immunity. The secondary injection of KLH included CA to sensitize calves to evaluate cell-mediated immunity (CMI; Table 6). *C. albicans* Strain 5314 was obtained from Tod Reynolds from the University of Tennessee. *C. albicans* was prepared following the protocol by Cárdenas-Freytag et al. (1999). *C. albicans* was incubated at 37°C overnight for 24 h. A yeast peptone dextrose agar plate was used for the incubation process. Cells were collected after 18 h and washed 3 times. Cells were counted (2×10^6 cells) and heat killed at 60°C for 2 h. Heat killing was confirmed through plating cells on yeast peptone dextrose plates 37°C overnight. Prior to intradermal injections, the right side of the calf's neck was shaved using 40 blade clippers (Andis, Sturtevant, WI). Cell mediated immunity was evaluated by conducting a delayed hypersensitivity test (DTH) to CA at d 28 ± 7 . Cell mediated immunity was tested by triplicate intradermal injections of CA (2×10^6 cells) and pyrogen free saline in the side of the neck. Skinfold thickness measurements to determine DTH to CA and saline was completed with digital calipers (Pittsburg Calipers, Calabasas, CA), and was conducted 0, 6, 24, 48 h relative to injections (Pollock et al., 1991). A stimulation index was calculated by mean CA response over mean saline injection responses, allowing for each calf to serve as its own control.

Algometry

At 35 ± 10 d after birth, calves were disbudded. Prior to disbudding, calves were given an IM injection in the neck of Xylazine (15 mg; IM, VetOne, MWI Animal Health, Boise, ID) followed by a corneal nerve block (6 mL at each disbudding site, 2% lidocaine; 240 mg, SC,

VetOne, MWI Animal Health, Boise, ID). Pain sensitivity to disbudding was evaluated using a pressure algometer (Mark-10 Corporation, Copiague, NY) containing a blunted rubber tip. The algometer was used at 4 different locations surrounding both disbudding sites (Heinrich et al., 2010). Measurements were taken -1, 3, 27, 51, and 75 h relative to disbudding (Mintline et al., 2013). When a calf expressed avoidance from pressure (ear flicking, tail flicking, head rubbing, head shaking, or foot stomping), the amount of pressure expressed in kilograms/second of force was recorded as the maximum tolerance (Heinrich et al., 2010).

Statistical Analysis

Separate mixed model analyses of variance (ANOVA) were performed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC). Normality was assessed using PROC UNIVARIATE for all dependent variables. The response variables of interest included ELISA units for IgG and IgM, STP, max response to CA, and ratio responses to CA and algometry. The fixed effects of housing treatment, time, and day were included in the analyses, as well as the interactions between these variables. The repeated measures of day and time of collection were included as random effects with first-order autoregressive correlation structure (AR1). Serum total protein values were placed into 1, 2, or 3 scores. A STP score of a 1 contained a value of 5.5, a STP score of a 2 contained values of 5.5 to 6.9, a score of a 3 contained values of 7.0 or greater. When appropriate to best fit the model, sex and birthdate were included as random effects. Backward manual elimination was used in model building, and AIC was assessed for model fit. All possible three way interactions were included in model building. LSmeans were reported for each variable of interest. Significance was determined at $P \leq 0.05$.

RESULTS

Thirteen calves were not enrolled or eliminated from the study due to the following: (1) birth defect; (1) failure of serum total protein test; (2) aged out (greater than 7 d of age) prior to implementing housing treatment; (9) euthanized for clostridium, heart murmur, and other genetic defects during the study. Surprisingly, one non-focal calf from a pair was euthanized due to clostridium, however the other calf remained unaffected. The focal calf was eliminated from the study due to losing its paired companion, however this calf remained healthy for the study observation period. Health scores were collected from 1 to 8 weeks of life following the score method of (Table 7; McGuirk, 2008). Mean fecal (Individual = 0.56 ± 0.97 SD vs. paired = 0.50 ± 0.84 SD) and respiratory scores (Individual = 1.23 ± 1.40 vs. paired = 1.06 ± 1.13 SD) did not differ between housing treatments.

No overall differences were found between housing treatments in response to CA (Figure 4; $P = 0.95$), however there was a time effect (Figure 4; $P < 0.0001$). A tendency was observed for individual calves to have a greater response at 48 h (Figure 4; $P = 0.08$). Calves in both housing treatments did not differ in maximal reaction sizes ($P = 0.83$; individual = 8.03 ; paired = 7.84 ± 1.15 cm). Serum total protein did not alter maximal size in preweaned calves ($P = 0.70$).

All calves regardless of housing treatment did not differ in STP ($P = 0.73$) and STP did not elicit a difference in IgM ($P = 0.58$) and IgG ($P = 0.97$) ELISA units to KLH. Therefore, no unintentionally blocking of calves by high and low responders to KLH occurred. No differences were found for anti-KLH IgM (Figure 5; $P = 0.78$). However, a day effect ($P < 0.0001$; d3 = 0.24 ; d7 = 0.15 ; d14 = 0.09 ; d35 = 0.09 ± 0.04 ELISA units) was observed. Additionally, calves that had a STP score of a 3 had higher IgM ELISA units ($P = 0.03$; STP1 = 0.10 STP2 = $0.12 \pm$

0.03 vs. STP3 = 0.21 ± 0.04 ELISA units). Anti-IgG KLH ELISA units did not differ between housing treatments (Figure 6; $P = 0.71$), however, for all calves, there was a day effect ($P = 0.0003$; d3 = 0.78; d28 = 0.73; d35 = 0.80 ± 0.04 vs. d7 = 0.57 ± 0.04 ELISA units).

Regardless of housing treatment, calves did not differ in sensitivity responses to the algometer after disbudding ($P = 0.24$, Figure 7). Left side ($P = 0.56$; individual = 3.12 vs. paired = 2.96 ± 1.19 kg of force), right side ($P = 0.58$ individual = $3.01 \pm$ paired = 3.17 ± 0.20 kg of force), and maximal ratio ($P = 0.94$; individual = 0.88 vs. paired = 0.89 ± 0.07 sensitivity ratio) did not differ between housing treatments. However, calf sensitivity ratios increased after h -1 (Figure 7; $P < 0.0001$) in all calves.

DISCUSSION

Previous literature has evaluated the humoral or cell mediated immunity of calves in social environments, however the aim of the current study was to measure both immune branches in preweaned calves housed in pairs or alone. Mitigating pain has successfully been accomplished through the use of NSAIDS, however the present study observed the effect social housing alone on reducing sensitivity in calves post disbudding. Regardless of housing treatment, calves were able to elicit an immune response, and pair housing calves did not negatively affect immune competence or pain sensitivity to disbudding. Colostrum played a significant role in the calves' ability to respond to KLH and should be considered in future studies when evaluating the immune development of young calves.

Calves in both housing treatments did not differ in IgG and IgM ELISA units. However, other housing environments of preweaned calves can influence antibody development. For instance, in early work, Cummins and Brunner (1990) evaluated calves housed in hutches rather

than metal pens at 42 and 56 days with KLH, and found that calves in hutches had greater IgG levels than calves reared in metal hutches, but this was not observed for IgM isotypes.

Furthermore, these concentrations increased with age. In the current study, calves at d 3 had just as high of IgG anti-KLH antibodies as days 28 and 35 of age. Our results differ from Cummins and Brunner (1990) due to calves in the previous study being deprived of colostrum, and immunity was assessed at 5 weeks of age. Calves in the current study were given high quality colostrum and immune evaluation began at 7 days of age, which may explain the difference in antibody development against KLH. Pollock et al. (1991) observed IgG to KLH of colostrum fed calves at 3 wks old to not be correlated with lymphocyte counts; however, this was not the case for the same calves at 5-months old when re-evaluated. These data from Pollock et al. (1991) suggest maternal antibodies early in life play as immunoglobulin mediators, and falsely represent antibody development in preweaned dairy calves. Additionally, the influence of maternal antibody protection decreases as calves get older.

We hypothesized that paired calves would increase specific antibodies to KLH after primary and secondary injections. However, in the current study naturally occurring antibodies from the colostrum were able to bind to KLH, even though dams producing the colostrum were naïve to KLH. We tested the colostrum against KLH, and colostrum had greater IgG ELISA units than d 3-calf serum and FBS across different dilutions (Figure 8). In previous work, natural antibodies recognize pathogens with similar shapes of KLH, which may have led to cross reactivity in the current study (Guigou et al., 1991; van Kneegsel et al., 2007). Calves were not deprived from colostrum, which may need to be considered in the future. For instance, calves born from cows without a dry period had greater antibody production in relation to calves from

dams with 30 and 60 d dry periods (Mayasari et al., 2015), therefore, by depriving colostrum, we may get a more in depth understanding of the calves' ability to respond to a novel antigen.

In the current study, calves with greater STP levels, had greater IgM ELSIA units. The colostral antibodies and antibodies present in calf plasma are highly correlated (Chase et al., 2008), and may explain why STP influenced IgM ELISA units. Unlike IgG, IgM ELISA units continued to decrease after d 14 of age. This may be explained by IgG being the primary immunoglobulin transferred from colostrum to neonatal ungulates, and IgM and IgA being the lowest antibodies transferred (Saya et al., 2016). We would speculate that IgG would be more positively associated with STP levels, however IgM antibodies were more associated with STP scores. In addition, it is important to note IgM concentrations have been falsely represented due to the non-specific binding to antigens prior to inoculations of KLH (Korver et al., 1984). Regardless, successful passive transfer of antibodies resulted in calves having more immunoglobulins against KLH, therefore it can be further argued calf immune competence is highly dependable upon colostrum absorption.

The neutralizing antibodies present in colostrum may have also impacted the calves' CMI to CA. Calves were inoculated with intradermal injections of heat-killed CA to evaluate CMI. Skin thickness was measured hours relative to injection to determine DTH. Calves DTH peaked at h 24, and remained constant until 48 h post injection. These data are in contrast to previous work with lactating dairy cows. Lactating cows peaked at hour 6 post CA injections, and significantly dropped by hour 24 (Heriazon et al., 2009a). The difference in DTH between these studies may be from dairy calves having a delayed ability to respond to antigens similar to adult cattle (Morein et al., 2002; Firth et al., 2007; Chase et al., 2008). However, it is suggested calves

can recruit immune cells, such as neutrophils, by 1 week of age (Kampen et al., 2006). Neutrophils may have been the immune cells recruited to injection sites, but biopsies were not collected in the current study to determine which cells types were present. Anecdotally, dermal responses were observed to decrease at h 96 and 120 in calves (data not shown), however Heriazon et al. (2009b) observed lactating cows significantly dropping response to CA by h 24. *Candida albicans* was successful at eliciting an immune response in preweaned calves, and a tendency for paired calves to decrease responses by 48 h may suggest these calves were able to recover their response to CA. Nevertheless, future studies should measure reaction sites at an extended period and take biopsies to evaluate CMI in preweaned calves.

Natural factors other than the environment, such as genetic backgrounds of the dam and sire, can influence the colostral protection in calves. For instance, previous literature has demonstrated that Simmental and Pinzgauer bulls tended to have lower IgG concentrations in relation to Hereford and Herford crossed sires (Norman et al., 1981). Therefore, it can be suggested that the Ig protection is heritable. One potential explanation is the heritability of a diverse major histocompatibility complex (MHC). The more diverse the MHC, the more antigens can be displayed on the surface for T and B cell activation and antibody production (Mallard et al., 1989). Although sera samples were collected from calves in the current study, genetic testing, like the MHC capacity was not analyzed. However, we were able to determine no unintentional blocking of calves for high and low responders towards KLH occurred. The average STP score for individually housed calves was 6.33 and 6.23 for paired calves. Therefore, all calves began the study without bias in responsiveness to immune stimuli.

Calves in the present study were injected with KLH and CA at an earlier age in relation to calves in previous work (Cummins and Brunner, 1990; Pollock et al., 1991; Mayasari et al., 2015). We chose to inoculate calves earlier in age to minimize influence of immunity that could have followed disbudding. Disbudding calves has previously elevated acute phase proteins, thus suggesting acute stress suppresses the immune response (Sylvester et al., 1998; Doherty et al., 2007). Calves in the current study were disbudded at 35 d of age, and the immune measurements ended prior to disbudding. Calves in the current study may have been given their primary injection too soon at 7 d of age, thus explaining the lack of reaction one week after the primary injection (14 d of age). Furthermore, the drop in IgG at 14 d age could be from the half-life of maternal antibodies and the finite protection during the first 3 weeks of life (Chase et al., 2008). Furthermore, the decrease in IgG at 14 d of age with the increase of IgG at 28 and 35 d of age may have been the window of susceptibility where calves were beginning to respond on their own to KLH. Therefore, age to measure immune competence and disbudding should be studied further.

Pain sensitivity did not differ between the housing treatments in the current study. However, social support in mammals has provided comfort to reduce discomfort behaviors. In humans, it is well documented social support reduces pain tolerance when evaluated on a 10 point scale (Brown et al., 2003). Group housed laboratory rats allow more painful shocks to be applied when approaching food and water resources in relation to individual rats, thus suggesting social support influences confidence to tolerate painful stimuli. (Morrison and Hill, 1967; Liu and Wang, 2005). Although this is true in other species, the disbudding in dairy calves may be too painful for social support to alter the pain threshold. Calves were less sensitive at h 3 than h

27, 51, and 75, however this could be from the lidocaine block and Xylazine not wearing off at that time. Pain mitigation techniques were successful in reducing pain sensitivity 3 h after disbudding, but housing treatment did not affect responses. In addition, it has recently been demonstrated pain from disbudding can lead to negative cognitive abilities. For example, in an attempt to measure cognition of young calves after disbudding, Neave et al. (2013) observed individual calves not given NSAIDS to struggle with reversal learning tasks in relation to calves that did receive NSAIDS. Therefore, it can be suggested that pain affects the ability for calves to cope with a changing environment. Future studies should evaluate pain in socially housed dairy calves, the use of NSAIDS, and immunity to determine if social contact in combination with these variables can minimize sensitivity to disbudding.

CONCLUSIONS

Individual and pair housed calves did not differ in adaptive or cell mediated immune responses, however all calves were able to elicit an immune response over time. Colostrum plays a major role in neonatal calf IgG and IgM development to KLH. Pain sensitivity to disbudding did not differ between individual and pair housed calves. These data suggest pair housing does not negatively impact calf immune competence or pain sensitivity to disbudding, therefore early socializing calves in a well-managed facility can be implemented without causing immune dysregulation.

CHAPTER IV
CONCLUSIONS

Getting dairy calves started off to a great start, or in other words, providing calves an environment that will allow them to grow and maintain health as early as possible, is the goal of every dairy producer. Growing the next generation to outperform the ones before is a mindset that only prosperity can grow from in the dairy cattle business. In order to do this, areas of interest in dairy calf management should be behavior, growth, and immunity.

Dairy calves are typically housed individually for the first 6 to 8 weeks of life to prevent the spread of disease and unwanted cross-suckling. Although these intentions were for the benefit of the calf, there are negative outcomes to this management style, such as poor cognitive functioning and the inability to adapt to novel environments. Dairy calves are gregarious animals, and have been for thousands of years. Therefore, it should be no surprise that there are benefits that can come from socializing calves earlier in life. In the current study, dairy calves were observed in an individual or pair housed pen. Resting posture, standing behaviors, play, and other behaviors were observed. Paired calves were observed to stand more often and spend more time awake, thus suggesting early pair housing encourages greater activity in the pen. However, more is needed to understand what behaviors calves should express in order to become more successful prior to weaning. For instance, behaviors are not constant for the entirety of the preweaning phase. Oral behaviors (licking the pen, eating straw, ingesting grain, etc.) all differ with age. In addition, literature is inconsistent with behaviors performed with calves housed individually and in pairs (DM intake and social play). Therefore, it is important for future studies to continue monitoring behavior of calves in a social environment and alone. The goal will be to understand what behaviors are critical during the early development of neonatal calves. Ideas in the future would be to switch housing treatment of calves after being acclimated to the social or

alone environments. This housing switch will allow further understanding of early social contact, and determine if previously paired calves seek companionship, or become accustomed to their new environment. In the current study, individual calves were observed to project their head out of the pen more often than pair housed calves, however it would be interesting to observe how that behavior changes when switching housing treatments. Another example would be to observe social hierarchy development of calves in a social environment. Calves grow and enter the lactating herd, where resource availability is limited. Allowing calves to be more competitive earlier in life may benefit calves long-term. Another important aspect to understand is the time budgets of dairy calves during the preweaning phase. It is understood that calves will lay down for > 17 h/d when on dry, clean bedding, but it is not understood how much lying time is important for calf development. Paired calves were observed to stand more often than individual calves in the current study, but all calves were able to lay down for roughly 18 h/d. Understanding lying behaviors, along with other time budgeted activities, can create bench marks for producers when raising calves. Finally, there are many studies that have observed benefits from early socialization during the preweaning and weaning period, but much is to be learned of the long-term benefits of early socialization. Future scholars are encouraged to observe calves after weaning, through heifer development, and into lactation.

Literature observing behavior and growth of dairy calves has previously provided promise. For instance, many studies claim social housed calves initiate social facilitation (one calf mimics another), thus they begin to increase grain consumption sooner. Other studies, however, claim that regardless of housing treatment calves grow the same. Calves in the current study were observed to grow at similar rates regardless of housing treatments. However, it

critical to account for the management practices of the current and previous studies. For instance, some fed calves high volumes of milk, and others only fed 10% of body weight or less. Calves in the current study did not differ in milk allowance throughout the entire preweaning phase, which may have played a role in our results. Future studies should evaluate at what level of milk allowance is beneficial for calves in a group setting that promotes increased growth, while encouraging calves to ingest grain. In addition, future studies should evaluate what milk volume level is necessary to mitigate health outcomes, i.e. scours and respiratory disease. In reference to health and milk allowance, it would be interesting to evaluate the difference between milk replacer and pooled, pasteurized milk on calf growth, outward health, and immunity.

The purpose to prevent calves from being socialized is the risk of disease outbreak. However, data on calf health is inconsistent. Recently conclusions state the health of calves is highly depended upon hygiene, treatment success, the presence of other species, and ventilation. In addition, results from the current study suggest calves given adequate amounts of good quality colostrum, there was no difference in immune development to novel antigens. Therefore, future research should focus on various environmental types and how that can impact calf immunity. Additionally, colostrum should be deprived in calves in future studies in order for the influence of maternal antibodies to be minimized on calf immune development to novel antigens. Future ideas can branch from previous work in order test these outcomes. For instance, calves can be housed in a social environment at various methods of ventilation, controlling the amount of antigen exposure (i.e., quarantined calving area and calf housing area, or implementing variations in biosecurity), and the age of novel antigen exposure to challenge immunity. Similar to behavior observations, the immune challenges should also go beyond the preweaning stage

and observe calves at weaning, during heifer development, and lactation to determine if social housing, along with various management practices during the preweaning stage, have long lasting impacts of immunity.

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APPENDIX

Table 1. Peer reviewed articles that have evaluated calf lying and standing behaviors on various bedding types.

Treatment	Animals	Bedding Type	Lying Time	Standing Time	Social Partner	Reference
Disbudding	Holstein, Holstein × Jersey	Straw	17.3 h/d	N/A	Individual	Black et al., 2017
Bedding DM	Holstein	Concrete with sawdust	18.0 h/d	N/A	Individual	Camiloti et al., 2012
Flooring type	Holstein	Concrete and mats	18.6 h/d	N/A	Group	Hänninen et al., 2005
Lying surfaces	Holstein & Jersey	Sand, gravel, or rubber	16.1 h/d Holstein; 15.2 h/d Jersey	N/A	Individual	Kurman et al., 2014
Milk allocation	Holstein	Sawdust	N/A	Restricted: 6.4 h/d; Ad libitum 5.5 h/d	Group	De Paula Vieira et al., 2008
Lying behavior & <i>Mannheimia haemolytica</i>	Holstein	Straw	14.6 h/d infected; 14.5 non-infected	N/A	Group	Eberhart et al., 2017
Individual vs. pair housing	Holstein	N/A	17.3 h/d individual; 16.6 h/d paired	Individual 47.4 min/d; Paired 69 min/d	Individual or paired	Chua et al., 2002
Environment & Temperature	Holstein	Straw	N/A	303 min/d	Individual	Hill et al., 2013
Social & Enrichment	Holstein	Straw	13.1 h individual; 9 h paired	3.7 h individual; 3.75 h paired	Individual or paired	Phillips et al., 2004

Table 2. Ethogram used to determine behaviors of calves in both housing treatments.

Behavior	Description	Housing Treatment	Citation
Cross suckling	The muzzle of a calf is suckling on any part of another calf.	Paired only	(De Passillé et al., 2010)
Licking pen	The calf's tongue is out of its mouth and in contact with the pen.	All calves	(Jensen and Bube, 2006)
Eating straw	Calf in oral contact with straw, followed by mastication.	All calves	(Heloape et al., 2006)
Head out of pen	The calf's head out of pen, not in grain or water bucket.	All calves	(Chua et al., 2002)
Calf proximity	Calf within a head's length of the muzzle of another calf were considered together. If calves were together and physically touching, they were together and touching. If the distance was more than a head's length, calves were alone.	Paired only	(Duve and Jensen, 2012)
Self-grooming	Tongue is out and in contact with own body.	All calves	(Cavestany and Eardenburg 2010)
Allogrooming	Tongue of one calf is out and in contact with another calf.	Paired Only	
Lying postures	Postures: "Long Resting", the calf lies on its sternum and ventral side of the abdomen with neck straightened. "Short Resting", the calf lies on its sternum and ventral side of the abdomen, curled up with the head turned back. "Wide Resting", the calf lies on its lateral side, hind legs stretched. "Narrow Resting", the calf lies on its sternum and on its lateral side of the abdomen, legs not stretched.	All calves	
Awake/Sleep	Awake: Eyes open, head lifted up, head moving actively, standing, or lying with head lifted and moving. Sleep: Resting head lifted and still, or resting neck relaxed, and muscle twitching may be present.	All calves	(Haanninen et al., 2008).
Play	The calf is engaged in a gallop, leap, bucking, or turning. The calf is standing; butting head against milk or water buckets, pen, or another calf in a playful manner.	All calves	(Pempek et al., 2016)
Social facilitation	One calf has head in the bucket of grain or water followed by another calf replicating the same behavior.	Paired only	
Ingesting grain	Head in the bucket and followed by mastication.	All calves	(Pempek et al., 2016)
Ingesting water	Head in water bucket drinking.	All calves	
Anticipation for milk feedings / hunger at mid-day and mid-night	Observation began 30 min before milk bottle delivery and ended 30 min after bottle removal, 1h at mid-day (10:30 – 11:30), and 1 h mid-night (21:00 and 22:00).	All calves	

Table 3. Least squares means of the percentage of time individual and pair housed calves spent in pen utilization behaviors, at early (16 – 18 d of age) and late (42 – 44 d of age) age observations.

		Proportion (%) of time observed							
Treatment		Paired	Individual			Early	Late		
Category	Behavior	Mean	Mean	SEM	P value	Mean	Mean	SEM	P value
Posture	Long	44.71	42.34	0.04	0.71	32.15	55.61	0.04	<0.0001
	Wide	0.80	0.50	0.009	0.67	0.61	0.66	0.003	0.71
	Narrow	5.90	3.40	0.02	0.43	6.39	3.11	0.02	0.07
	Standing	26.9	26.23	0.02	0.8	27.74	25.42	0.02	0.21
Awake/Sleep	Awake	76.21	70.33	0.02	0.03	64.17	80.91	0.02	<0.0001
	Sleep	5.88	4.32	0.02	0.58	4.88	5.22	0.01	0.76
Play	Locomotor	0.35	0.43	0.001	0.63	0.35	0.44	0.001	0.53
Feeding	Grain	3.18	2.21	0.004	0.13	2.18	3.38	0.003	<0.0001
	Water	0.94	0.71	0.002	0.42	0.83	0.8	0.002	0.88
	Milk	1.81	2.15	0.001	0.11	2.28	1.7	0.001	0.01
Oral	Licking Pen	2.74	4.16	0.01	0.15	2.42	4.68	0.01	0.002
	Eating Straw	2.12	2.27	0.005	0.83	3.10	1.55	0.004	0.003
Social	Self-grooming	3.83	4.44	0.003	0.26	3.5	4.86	0.003	0.01
	Head out of Pen	1.37	2.75	0.003	0.02	2.58	1.46	0.003	0.01

SEM= Standard error of the

Table 4. Least squares means of the percentage of time individual and pair housed calves spent in behaviors at feeding observations. These behaviors were observed at 1 min scan sampling. Calves were observed at early (16 – 18) and late (42 – 44) ages.

Proportion (%) of time observed								
Treatment	Paired	Individual			Early	Late		
Behavior	Mean	Mean	SEM	<i>P</i> value	Mean	Mean	SEM	<i>P</i> value
Lying	65.86	73.21	0.04	0.24	68.18	71.1	0.03	0.24
Grain	2.85	2.42	0.01	0.75	3.45	1.99	0.01	0.04
Licking Pen	2.96	6.00	0.01	0.13	4.98	3.58	0.01	0.16
Eating Straw	4.04	3.43	0.01	0.66	2.52	5.46	0.004	< 0.001
Self-grooming	3.21	4.44	0.01	0.17	4.43	3.97	0.01	0.6
Head out of Pen	1.68	2.02	0.01	0.72	2.06	1.64	0.005	0.4

SEM= Standard error of the mean

Table 5. Least squares means of the percentage of time calves spent in anticipation and hunger behaviors at specified feeding times. PRE (30 minutes before bottle delivery at AM and PM feedings), MD (10:30 – 11:30), POST (30 minutes after bottle delivery at AM and PM feedings), and MN (21:00 – 22:00) were the times observed over 3 consecutive d. The *P* value represents a difference between observation time points.

Behavior	Proportion (%) of time observed			
	Observation	Mean	SEM	<i>P</i> value
Lying	PRE	29.84 ^a	0.04	< 0.001
	MD	87.89 ^b	0.03	
	POST	57.39 ^c	0.04	
	MN	87.99 ^b	0.03	
Grain	PRE	5.07 ^a	0.01	0.0014
	MD	1.32 ^c	0.01	
	POST	3.71 ^{ab}	0.01	
	MN	1.88 ^{bc}	0.01	
Licking Pen	PRE	7.70 ^a	0.02	0.0008
	MD	2.41 ^c	0.01	
	POST	5.52 ^{ab}	0.01	
	MN	3.05 ^{bc}	0.01	
Eating Straw	PRE	2.51 ^a	0.01	< 0.001
	MD	4.54 ^c	0.09	
	POST	6.83 ^b	0.01	
	MN	2.42 ^a	0.01	
Self-grooming	PRE	4.60 ^a	0.01	0.75
	MD	4.77 ^a	0.01	
	POST	3.56 ^a	0.01	
	MN	3.96 ^a	0.01	
Head out of Pen	PRE	12.7 ^a	0.002	< 0.001
	MD	0.58 ^c	0.002	
	POST	2.64 ^b	0.007	
	MN	0.53 ^c	0.002	

^{a-c} Represents difference in means between each observation time observed.

SEM= Standard error of the mean.

Table 6. Day ($\pm$ SE) of age, injected antigen, volume, and blood collection status is recorded below. These were efforts to evaluate antibodies against keyhole limpet hemocyanin (KLH) and delayed type hypersensitivity (DTH) to *Candida albicans* (CA).

Age (d)	Injected Antigen	Volume (mL)	Blood Collection
3 $\pm$ 2	--	--	Yes
7 $\pm$ 3	KLH	1	No
14 $\pm$ 3	--	--	Yes
21 $\pm$ 3	KLH	1	No
28 $\pm$ 3	DTH to CA	0.1 $\times$ 3	Yes
35 $\pm$ 3	--	--	Yes

' $\times$ 3' refers to 6 total injection sites.

Table 7. Average health scores and high score incidences for individual and pair housed calves. Numbers represented are absolute values.

Average Health Score			
	Resp	Fecal	
Individual	1.20	0.56	
Paired	1.10	0.50	
Incidences of high score			
	Resp =4	Resp=5	Rep=6
Individual	7	3	4
Paired	2	2	0
	Fecal=2		Fecal=3
Individual	20	13	
Paired	20	5	

Respiratory (Resp) and Fecal scores were recorded.

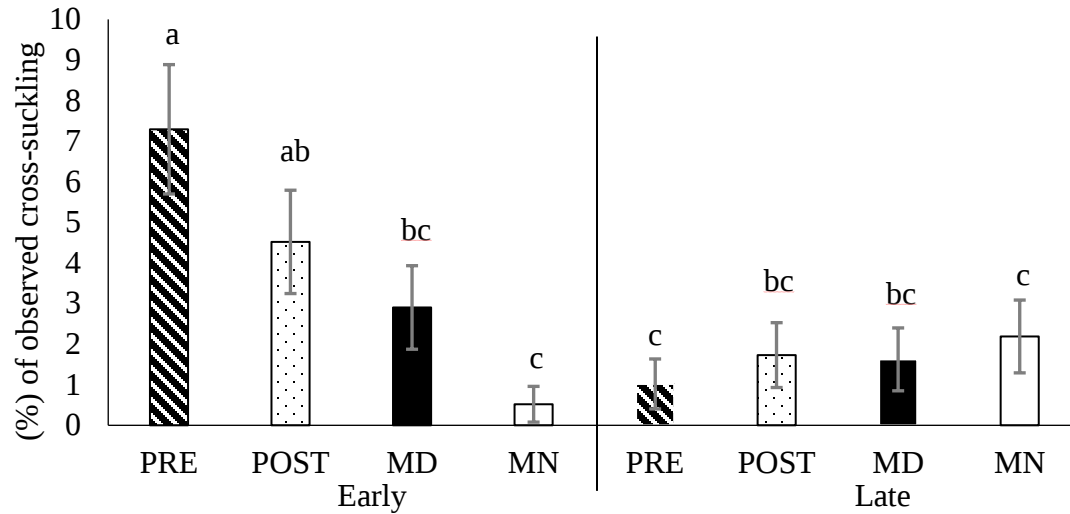


Figure 1. Age and feeding time interaction for cross-suckling was observed in paired calves ($P = 0.03$). Calves were observed at 2 ages (16 – 18 = early vs. 42–44 = late) for 3 consecutive d. Observations occurred at PRE (before bottle at AM and PM feeding), POST (after bottle at AM and PM feeding), MD (10:30 – 11:30), and MN (21:00 – 22:00) to determine hunger and anticipation behaviors of paired calves. ^{a,b,c}Letters represent significance between observation periods and age interaction.

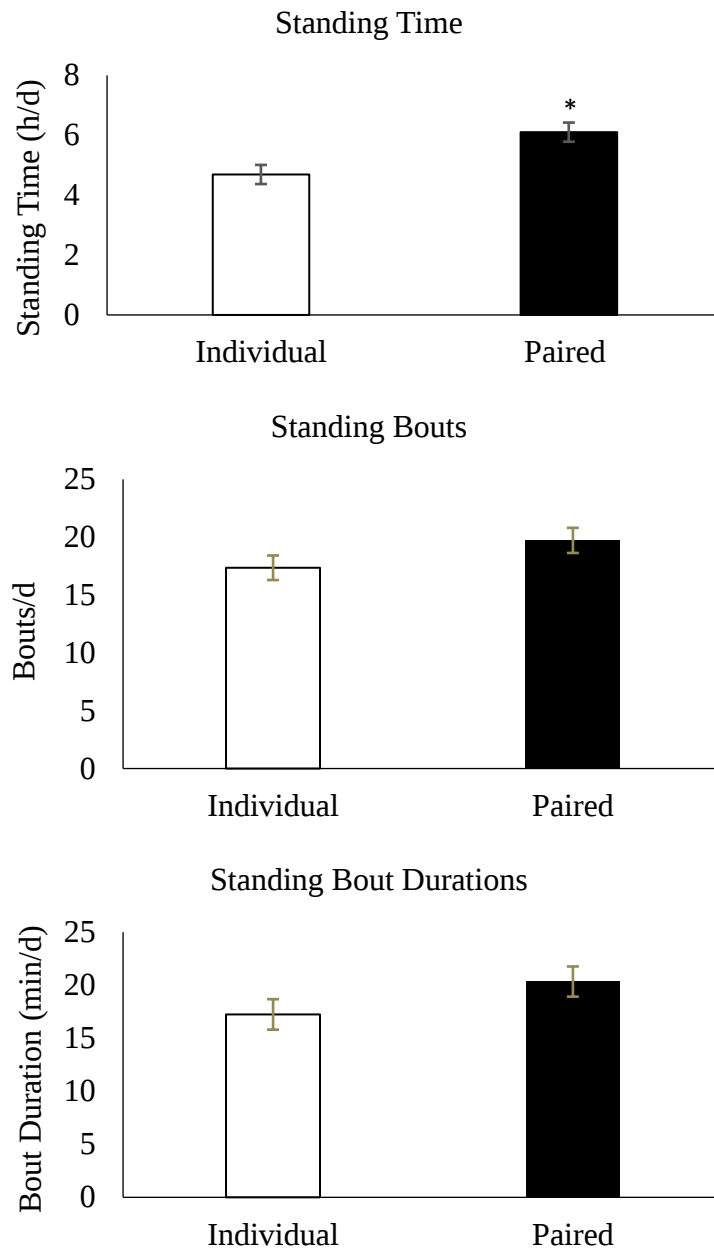


Figure 2. Paired calves had greater standing time than individual calves ($P = 0.004$). Difference in standing time is represented by (*). A tendency for paired calves to have greater bout duration was also observed ($P = 0.08$). Bouts did not differ between housing treatments.

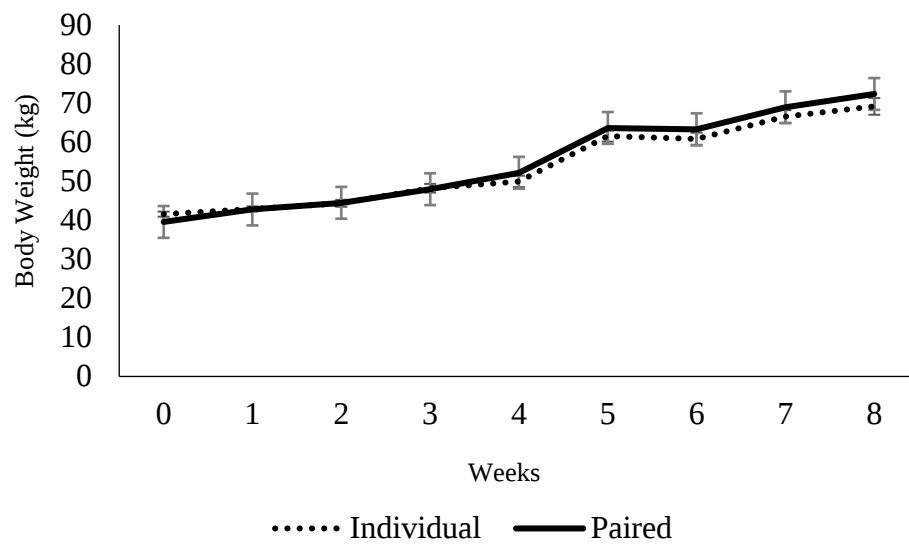


Figure 3. Weekly body weights (kg) of individual and pair housed calves from birth until weaning.

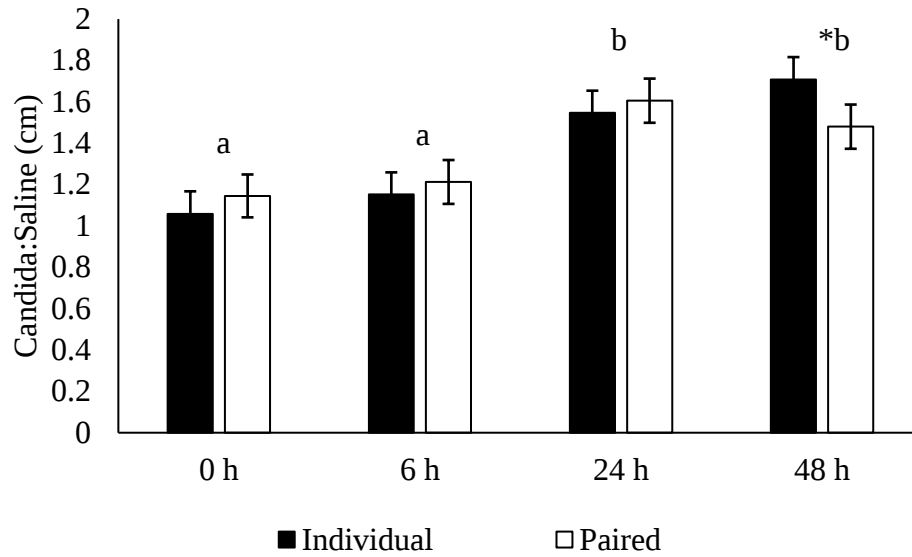


Figure 4. Responses to *Candida albicans* at h relative to intradermal injections. Calf responses began to increase after 6 h ($P < 0.0001$). ^{a,b}Letters represent significant differences in time. A tendency for individual calves is represented by (*) to have a greater response at h 48 in relation to paired calves ($P = 0.08$).

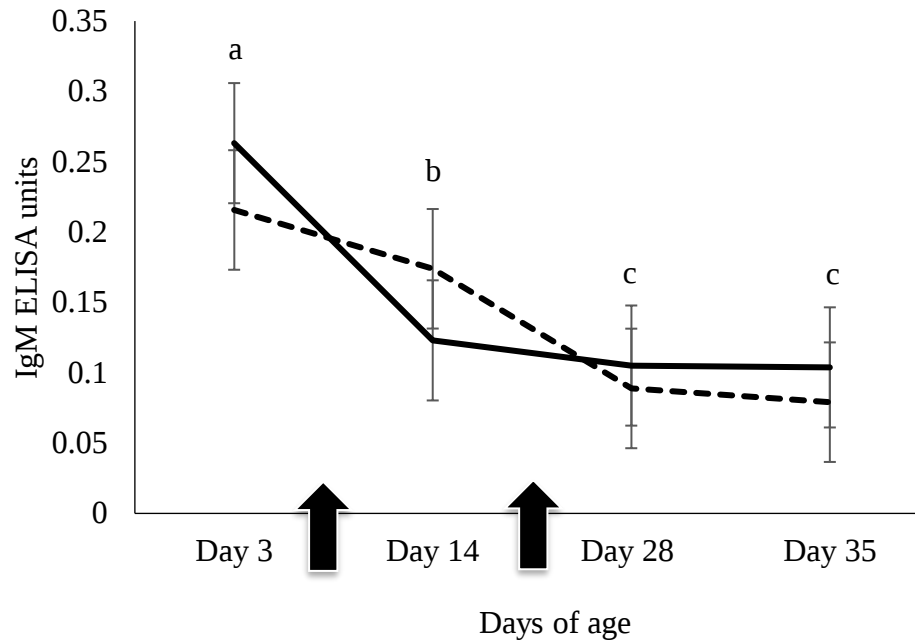


Figure 5. IgM ELISA units of individual (solid line) and paired (dashed line) calves at d 3, 14, 28, and 35 d of age. Black arrows represent the primary and secondary injection of keyhole limpet hemocyanin. ^{a,b,c}Letters represent significant differences between d ($P < 0.0001$).

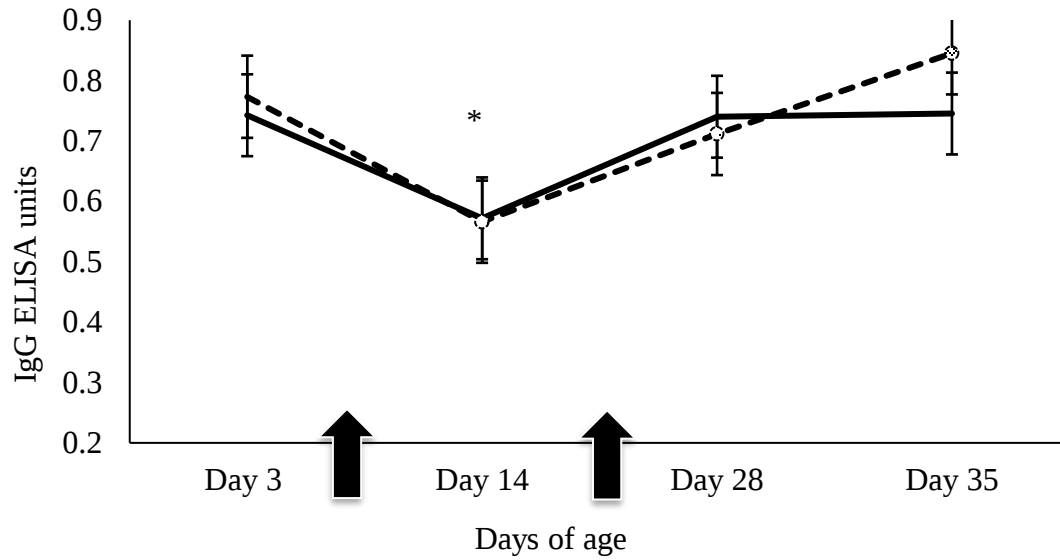


Figure 6. IgG ELISA units of individual (solid line) and paired (dashed line) calves at d 3, 14, 28, and 35 d of age. Black arrows represent the primary and secondary injection of keyhole limpet hemocyanin. The (*) represents the difference at d 14 in relation to other days ($P = 0.0003$).

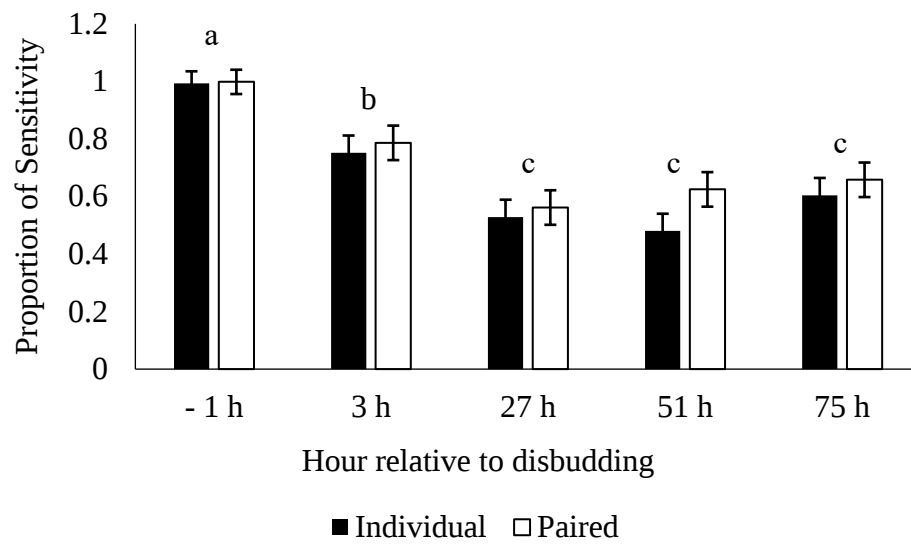


Figure 7. Mean proportion of pain sensitivity responses of individual and pair housed calves to the algometer in relation to disbudding time. ^{a,b,c}Letters represent significance over time ($P < 0.0001$).

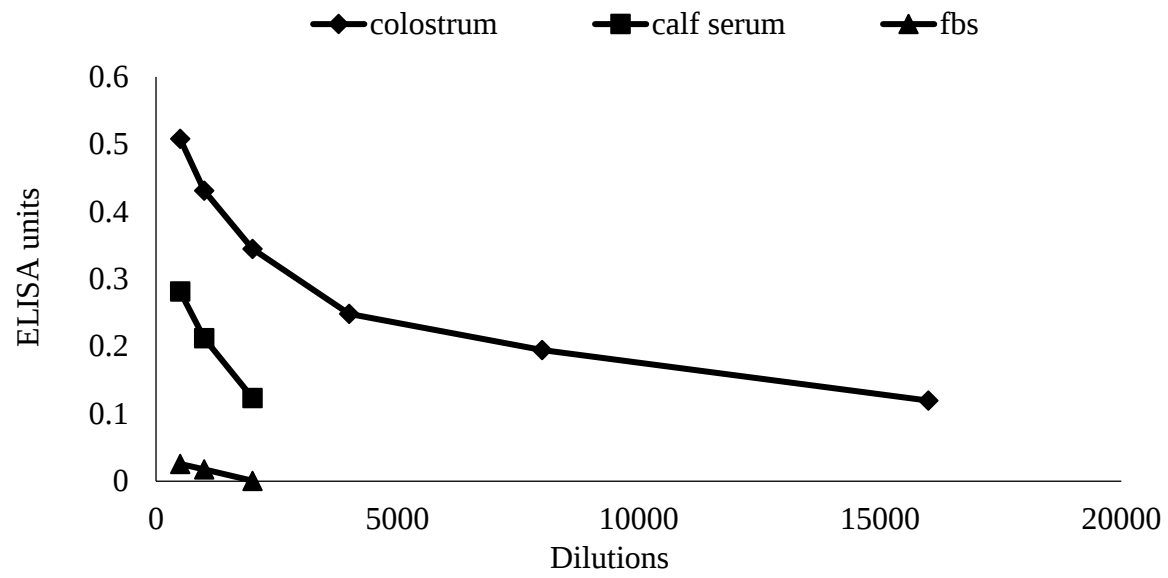


Figure 8. Calf d 3 of age (no injection of keyhole limpet hemocyanin), colostrum, and fetal bovine serum (negative control) ELISA units across different dilutions.

VITA

Clay Kesterson was born on February 9, 1993 in Morristown, TN. Clay graduated from Morristown Hamblen High School East in 2011 and continued his education at Walters State Community College from 2011 to 2013. Clay transferred to the University of Tennessee, Knoxville and gained his Bachelor's degree in 2016. Clay began his Master's degree in Fall 2016. During Clay's MS degree, he was active in Extension, worked on a classified project, went to professional development events, and attended international and regional conferences in reference to dairy cattle welfare and immunity. Clay also managed up to 15 undergraduates during his thesis for data collection on farm, and video observation off farm. Clay prefers to continue working within the dairy industry, however he is open to any positions in agriculture that are available. Clay has a passion for agriculture, and wants to work as hard as he can to be an advocate for all farmers around the world!