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The Effect of Bedding Surface and Muscid Fly Populations on the Welfare of Pre weaned Holstein and Jersey Calves

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To the Graduate Council:

I am submitting herewith a thesis written by Christa Anne Kurman entitled "The Effect of Bedding Surface and Muscid Fly Populations on the Welfare of Pre weaned Holstein and Jersey Calves." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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The Effect of Bedding Surface and Muscid Fly populations on the Welfare of Pre weaned Holstein and Jersey Calves

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ABSTRACT

The first objective of this study was to determine the effect of bedding on the welfare of Holstein and Jersey calves housed using individual hutches bedded with gravel, rubber mats, or sand. A second objective was to determine the effects of stable and house fly populations on calf welfare. Bedding quality and fly management are aspects of improving animal welfare on dairy farms. It was hypothesized that sand or mat would increase lying time and decrease lying bouts, but not affect BW gain or feed intake in calves indicating that these bedding surfaces increased calf welfare. It was hypothesized that as stable and house fly populations increased calf lying time and BW gain would decrease and plasma cortisol concentrations would increase. Twenty-three Holstein calves and 38 Jersey calves were assigned to 1 of 3 bedding treatments (gravel, rubber mat, or sand). Lying time and lying bouts were assessed with dataloggers. Biological function was determined by weight gain and grain intake. Plasma cortisol levels were determined using a commercial RIA kit. Data on the effect of bedding were analyzed using a mixed model in SAS with repeated measures. There was an effect of breed for all response variables so the two breeds were analyzed separately. Data indicated that there were no differences in overall calf welfare among bedding treatments. For the final analysis of the effect of stable and house fly populations on calf welfare 11 Holstein and 19 Jersey calves born between from September 2012 to October 2012 were utilized. Fly populations were monitored using alsynite traps. Data on the effect of stable and house flies on lying time, BW gain, and plasma cortisol levels were analyzed using linear regression in SAS. Data indicate that as stable and house fly populations increased welfare of calves decreased.

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INTRODUCTION

Animal welfare was defined by asking questions such as: 1) was the animal able to live a relatively natural life, 2) could the animal function well in its environment, and 3) what was the affective state of the animal, or how did the animal feel (Fraser, 2008). All aspects of welfare are interrelated; for example, illness can cause an animal to have decreased growth (biological function) and also cause the animal to feel sick (affective state; (Johnson and Borell, 1994). Furthermore, managing animals for only one aspect of welfare may not be effective. An example of this was that housing dairy calves in hutches reduced disease transmission (influenced biological function and affective state) but prevented calves from participating in natural social behaviors (influenced natural living; (Jensen, 2003). Housing and management strategies could affect the natural living, biological function, and affective state of dairy calves. Natural living involved the ability of the animal to behave in a manner similar to when it was in a natural environment, even when housed in confinement (von Keyserlingk et al., 2009). Dairy calves are often housed individually and fed milk twice a day, this was in contrast to when they were in a natural state where calves lived with their dams and conspecifics and had many small milk meals per day (von Keyserlingk et al., 2009). Goals for calf housing in relation to natural living included: providing calves with space to lie down, stand up, and turn around as well as providing them with a bedding surface which was soft, non-slip, and easy to clean (Webster, 1984a). Biological function guidelines in calves were based on the fact that in order for calf raising to be profitable, dairy calves must be managed to maximize weight gain and solid feed consumption while reducing morbidity and mortality (Webster, 1984a). High rates of calf morbidity and mortality were an animal welfare and economic concern for farmers (Mintline et al., 2013); (Waltner-Toews et al., 1986). Another area of concern was that dairy calves may experience a negative affecting state while undergoing painful procedures such as disbudding early in life (Mintline et al., 2013).

A major aspect of management which affected the natural living, biological function, and affective state of calves was fly pressure. The four major flies affecting dairy animals were stable flies (*Stomoxys calcitrans L.)*, house flies (*Musca domestica L.),* and horn flies (*Haematobia irritans L*.**;** Christensen, 1982). Fly pressure was an issue closely tied to housing and management because flies develop in manure, including older manure left in sheltered areas such as under gates and decaying organic matter, materials which are abundant on dairy farms (Dougherty et al., 1993). Common fly development locations included spilled feed, winter round bale feeding sites, and calf hutches (Todd, 1964; Schmidtmann, 1991). When calf hutches were bedded with straw, a single hutch could produce between 25,000 and 40,000 stable flies in one summer (Schmidtmann, 1988). Flies can dispersed to other areas of the farm, causing irritation to cattle and farm workers (Christensen, 1982).

Stable and house flies were two of the most serious pests on dairy farms in Tennessee (Stewart, 2007). Stable flies fed on blood by biting the lower legs of cattle, and their populations in Tennessee were highest from March to May

(Taylor et al., 2012; Stewart, 2007). The stable fly life cycle from egg to adult took from 2-9 wk and they developed in areas where manure was mixed with organic matter (Christensen, 1982). Stable flies were easily identified by their bayonet-like piercing mouthparts. When feeding on pastured or confined cattle stable flies could be found on the lower legs and underbellies of cattle often facing upward parallel to the cows' legs (Christensen, 1982). Chemical sprays applied to animals were unsuccessful in controlling stable flies because they were difficult to apply to the lower legs of animals and could be easily washed off if animals walked through water or vegetation (Christensen, 1982). It was recommended that stable flies be controlled on dairy farms using cultural control. Methods of cultural control for stable flies included removing breeding sites by cleaning up spilled feed, old manure in areas such as under gates, and round bale feeding sites (Stewart, 2007).

House flies did not bite; however, they could still irritate cattle by feeding on secretions from eyes and mucous membranes (Christensen, 1982). House flies also spread bacteria which could cause diseases such as conjunctivitis and mastitis (Christensen, 1982). Peak season for house flies in Tennessee was from April to October and may have extended as late as December if temperatures were mild (Stewart, 2007). The lifecycle of the house fly from egg to adult took from 1-2 wk and they developed in areas where manure was mixed with organic matter as well as in garbage dumpsters and other areas where organic matter and moisture were present (Christensen, 1982). House flies could be identified by their sponging type mouthparts and the yellow-orange color between their eyes (Geden et al., 2010). House flies were found around the eyes and mucous membranes of confined animals and were also prevalent in indoor spaces such as milk handling facilities (Stewart, 2007). Control of house flies could be achieved by removing breeding sites (cleaning up spilled feed, manure, round bale feeding sites and removing garbage), additionally, house flies could be excluded from animal areas as well as milk handling facilities using screens and windows (Geden et al., 2010). House flies could also be controlled with appropriately labeled premises sprays and application of insecticides to animals using back rubbers or ear tags (Stewart, 2007).

One major way to help control both stable and house flies on dairy farms might be adjusting calf bedding materials when fly season was at its peak. Calf hutches bedded with organic materials were a prime location for stable and house fly development (Schmidtmann, 1988). Utilizing an inorganic bedding material during peak fly season was an excellent way to reduce stable and house fly populations. Use of concrete mix sand bedding reduced stable and house fly larvae by 76% when compared to straw, while bedding calf hutches with pea gravel reduced house and stable fly larvae by 99% when compared to straw (Schmidtmann, 1991). In colder areas or places where sand and gravel are prohibitively expensive or not readily available, sawdust could be used because it reduced stable and house fly larvae by 46%. Sawdust may also have increased calf cleanliness when compared to sand or gravel because it provided an absorbent surface (Schmidtmann, 1988). In addition to bedding type, bedding moisture level and manure content also affected fly development. House fly larvae had higher survival and development in sand bedding containing higher levels of manure solids and moisture in a laboratory setting (Hogsette, 1996). In a field setting for every 1 cm of rainfall during late February through March one additional adult stable fly per front leg was observed during leg counts on adult cattle (Mullens and Peterson, 2005). This indicated that additional moisture may have increased stable fly populations on dairy farms and that it was important to maintain animals in a clean, dry environment in order to reduce fly populations. It was also important to monitor fly populations to determine the effects of management changes (such as a change in calf bedding material) and whether additional measures needed to be used (such as use of a chemical insecticide).

Two ways of monitoring fly populations (especially stable flies) on dairy farms were 1) performing leg counts and 2) using alsynite traps. Leg counts involved counting the number of stable flies on the front legs of at least 15 calves. If average stable flies on both front legs was greater than 10, economic losses such as decreased weight gain may result and fly management was needed (Gerry et al., 2007). This method was quick, easy, and did not require any special equipment. Alsynite traps used a translucent material that was attractive to stable flies covered with a sheet of sticky material to trap flies (Taylor and Berkebile, 2006). Traps were useful in providing information about fly population size when placed at the four corners of a given area such as a calf housing location (Kaufman et al., 2005).

Horn flies and face flies were different from stable and house flies in that they were most often found on animals housed on pasture and developed exclusively in fresh manure (Stewart, 2007; Geden et al., 2010; Christensen, 1982; Bruce, 1938). Horn flies were a small blood feeding fly common on cattle on pasture (Bruce, 1938). Horn flies caused a significant amount of stress and discomfort to animals and decreased feed intake and weight gain in pastured cattle (Loftin and Corder, n.d.). Peak season for horn flies in Tennessee was from May to September (Stewart, 2007). The horn fly lifecycle from egg to adult took from 2-3 wk and they developed exclusively in fresh manure (Stewart, 2007; Geden et al., 2010). Adult horn flies were much smaller than stable or house fly adults and had piercing mouthparts and prominent dark red eyes, these flies were most often seen along the back and sides of pastured cattle (Christensen, 1982). Because horn flies bred only in fresh manure cultural control which was effective for stable and house flies did not reduce horn fly populations. Horn flies could be controlled by application of appropriately labeled pesticides applied using sprays or back rubbers (Geden et al., 2010). Another method of controlling horn flies was using a walk-through type trap to remove the flies from cattle, because horn flies are an obligate parasite they died shortly after being physically removed from the host animal (Geden et al., 2010; Bruce, 1938).

Face flies fed on secretions from the eyes and mucous membranes of cattle as well as around wounds. They could transmit diseases such as pinkeye as well as cause a great deal of annoyance and irritation to animals (Geden et al., 2010). Face fly populations in Tennessee were highest from April-June and their lifecycle from egg to adult took 2-3 wk (Stewart, 2007; Geden et al., 2010). Like horn flies, face flies only developed in very fresh manure (Christensen, 1982). Face flies were identified by the sponging type mouthparts as well as the silvery white color between the eyes (Stewart, 2007). Face flies were found feeding around the eyes and mucous membranes of animals on pasture, and they rarely were found on confined animals as they would not enter darkened buildings (Christensen, 1982). Similar to horn flies, methods used to control stable and house flies were generally ineffective at controlling face flies (Stewart, 2007). Application of insecticides using sprays, back rubbers, or ear tags were successful at reducing face fly populations, providing animals with a darkened shelter was also helpful in reducing face fly feeding (Geden et al., 2010).

Although stable, house, horn, and face flies were all significant pests of dairy cattle, our study focused on the effects of stable and house flies as these were the most likely to affect confined dairy animals and calves are often housed in confinement. Calf hutches also provided an ideal environment for the development of both stable and house flies.

Natural living and behavior

The effect of housing and management on natural living

Animal welfare was an important concern for dairy calves because these animals were often housed individually in hutches or stalls, which were generally considered unnatural settings and could be a source of negative public perception of the dairy industry (von Keyserlingk et al., 2009). The majority (around 75%) of calves in the United States were housed in individual pens or calf hutches during the pre-weaning period (USDA, 2007). Calf hutches were widely used as a means to reduce the spread of disease and prevent cross suckling in pre-weaned calves (Kung et al., 1997). Other individual housing systems for calves included outside pens or indoor stalls (Dellmeier et al., 1985). Another important aspect of calf housing was bedding surface because it increased natural behaviors such as lying behavior bedding may also have increased weight gain in calves housed in cold environments because it insulated calves from cold flooring (Camiloti et al., 2012; Anderson et al., 2005). The ideal bedding surface for calves was soft, inexpensive, non-slip, and easy to clean and aided in keeping calves safe, clean, and dry (Webster, 1984a).

The effect of housing and management on lying behavior

Calf housing systems could negatively or positively influence the natural living and behavior of dairy calves. An example of an essential behavior in adult dairy cattle was lying behavior. Lying behavior was considered an important indicator of cow comfort and decreased in environments that were aversive to cattle, such as housing areas with restrictive stall hardware or hard floors (Haley et al., 2000). Cows placed a high priority on lying behavior, as they chose to lie down over eating or socializing when deprived of all of these behaviors, deprivation of lying was also stressful to cattle indicated by an increase in stress hormones (Munksgaard et al., 2005). Lying behavior was important in improving performance in lactating dairy cattle as lying increased mammary uterine blood flow which was an important factor in milk production and reproductive performance (Haley et al., 2000; Nishida et al., 2004). Lying behavior was likely an important indicator of welfare for dairy calves as well.

Calves spent 70-80% of each day lying down and calves with decreased lying time associated with decreased growth (Chua et al., 2002; Mogensen et al., 1997). A hard bedding surface in calf hutches affected lying behavior in dairy calves. For example, 5 wk old calves reared on sawdust (a soft surface) spent more time lying than calves housed on river stones (a harder surface; (Sutherland et al., 2013).

Most research involving the effect of calf bedding surface on calf lying behavior has been conducted during the spring and summer, which presented a need for research on the impact of bedding on behavior during winter months. Cold stress occurred in calves when they need to expend energy in order to maintain their internal body temperature. If calves are not provided with adequate nutrition during periods of cold stress, weight loss and increased illness could occur (Webster, 1984). The addition of bedding helped calves cope with cold stress by providing insulation from cold floors and therefore reducing heat loss by conduction with cold flooring (Webster, 1984).

The effect of fly pressure on lying behavior

Stable flies caused discomfort for cattle housed on pasture as well as in confinement (Todd, 1964). Cattle exposed to high stable fly populations exhibited avoidance behaviors such as bunching, kicking, and stomping, which were indicative of pain and annoyance (Todd, 1964). Cattle also stood in water to protect their legs and stomachs from bites (Todd, 1964). Participation in fly avoidance behaviors took energy and reduced the amount of time animals spent lying down. When dairy cattle herds on pasture were observed to have greater than 15 flies per cow the level of irritation and pain was sufficient that cattle made no attempt to lie down (Todd, 1964). Stable fly pressure also influenced the areas where dairy cattle chose to lie down, with cattle spending more time lying in manure in attempts to protect their legs and stomachs from flies (Vitela et al., 2006a). Although it was known that fly pressure influenced behavior in adult cattle, the effect of fly avoidance behaviors on aspects of natural living such as lying behavior in dairy calves has not been examined.

The effect of housing and management on social and play behavior

Social behavior was another important component of natural living for dairy calves. Calves were naturally social animals; in fact, individual housing of calves older than 8 wk was banned in some European countries (von Keyserlingk et al., 2009). Group housing was a possible alternative to individual housing and provided several benefits to calves besides facilitating social behavior. Calves reared in groups gained more weight and consumed hay and starter earlier than isolated calves, possibly due to group learning (Warnick et al., 1977). Group housed calves learned to compete for feed prior to weaning, which reduced the post weaning decrease in weight gain commonly seen with calves housed individually (Chua et al., 2002). Group housing may have allowed for more natural feeding behavior in dairy calves. In natural settings, calves stayed close to their mother and suckled many times per day (Fraser, 2008). In contrast, on many dairy farms calves were removed from cows within 24 h and fed milk from a bottle or pail twice/d (Fraser, 2008). This method of feeding led to increased cross suckling, or non-nutritive suckling of another calves' body or udder. This behavior was undesirable because it led to udder damage in heifer calves and, therefore, decreased production later in life (Keil et al., 2000). The fact that cross suckling decreased significantly after calves were weaned from milk indicated that this behavior can be stimulated by ingestion of milk (Lidfors, 1993). Feeding calves with an open bucket did not allow calves to satisfy their instinct to suckle and could increase cross suckling. In order to reduce cross suckling, calves could be fed with an artificial teat. This gave them a natural outlet for suckling behavior even after the milk meal was finished (Jensen, 2003). Group housing also allowed calves more space than individual housing to practice natural locomotor behaviors such as play (Jensen and Kyhn, 2000).

Play behavior in calves was not only an aspect of natural living; it could help calves prepare for stressful situations later in life (Spinka et al., 2001). Since calves were only motivated to play when their basic needs for nutrient intake and housing space were met, play behavior was an excellent indicator of animal welfare and could be used to evaluate the efficacy of housing and management systems (Krachun et al., 2010; Jensen and Kyhn, 2000). Play behavior could be evaluated with an open field test to show if a “damming up” response was present. The “damming up” response occurred when animals were released from confinement and expressed behaviors that were restricted during the confinement period, mostly those related to movement or defense (Lorenz, 1981). This response occurred when animals that were more confined (for example, calves in stalls or pens) showed more play and locomotor behavior than less confined animals (calves housed in hutches or yards; Dellmeier et al., 1985).

Play in young mammals provided training for unexpected situations that arise throughout the animal’s life. This was particularly relevant to dairy animals when they faced possibly stressful circumstances such as entering the milking parlor or eating through headlocks when they join the lactating herd (Spinka et al., 2001). Play behavior was also useful in determining the efficacy of pain abatement protocols (Mintline et al., 2013). Calves disbudded with a hot iron or “sham” disbudded received no treatment or were treated with local anesthetic, or local anesthetic and meloxicam (an analgesic). Sham disbudded calves spent more time playing than disbudded calves. Calves disbudded with no pain control spent less time playing than any other group of calves, indicating that the pain relief protocol was effective (Mintline et al., 2013). The results of this study supported two important concepts 1) animals in a compromised state showed reduced play behavior and 2) administration of pain control during and after disbudding appeared to decrease pain as indicated by no decrease in play behavior following the procedure (Mintline et al., 2013). Besides being an important aspect of natural living for dairy calves, play behavior indicated a positive affective state in response to the environment provided for the calf (Dellmeier et al., 1985).

Biological function

One of the most studied aspects of animal welfare was biological function, or how the animal performs and produces in a given environment (Fraser, 2008). Important goals for biological function in dairy calves included optimized weight gain, increased solid feed intake, and reduced morbidity and mortality (Warnick et al., 1977). Weight gain in calves was optimized to allow heifer calves to reach puberty as soon as possible; however, this weight gain must be carefully managed to prevent deposition of fat in the mammary gland, which could decrease milk production later in life (Davis Rincker et al., 2011). Calves should eat a high-quality starter ration as early as possible in order to reduce costs associated with providing milk replacer and to allow for early rumen development, which increased feed efficiency (Berends et al., 2012). An accelerated feeding program was an alternative to encouraging calves to eat starter grain. Accelerated programs involved feeding calves a higher amount of nutrition from milk replacer, which caused less grain consumption. Accelerated feeding programs increased body weight gain from birth to weaning when compared to a more traditional feeding program (Kmicikewycz et al., 2013). Although calves on accelerated feeding programs consumed less calf starter, they gained weight faster and were larger at weaning, additionally these calves had compensatory calf starter intake at weaning which allowed for rumen development (Cowles et al., 2006).

Calf growth should be around 0.4-0.6 kg/d during the pre-weaning period in order for calves to reach puberty by 10 months of age (Davis Rincker et al., 2011). Rapid growth during the post-weaning period or when calves are 3-10 months old should be avoided to reduce the risk of over-conditioned heifers or deposition of fat in the mammary gland, which could negatively impact milk production when the animal entered the lactating herd (Davis Rincker et al., 2011). Increased nutrient intake during the pre-weaning period, especially the intake of nutrients from milk replacer, could increase milk yield during the first lactation (Van Amburgh, 2008). This could be achieved using an accelerated feeding program, as mentioned previously, these programs involved feeding a high-protein milk replacer at an increased amount relative to the body weight of calves. Calves fed a conventional milk replacer gained 0.45 kg/d, and calves fed a larger amount of accelerated milk replacer with higher fat and protein than the conventional diet gained 0.68 kg/d. Calves fed the accelerated diet were younger at the time of conception and at first calving than conventionally fed calves with no difference in cost of raising calves or first lactation milk production (Davis Rincker et al., 2011). It was important to note that there were a variety of calf feeding protocols and that the accelerated program was one way of optimizing calf growth (Davis Rincker et al., 2011).

Another important aspect of biological function in dairy calves was the early consumption of a calf starter ration. Provision of solid feed was essential for transitioning the calf from a pre-ruminant to a ruminant animal (Miller-Cushon et al., 2013). Calves should be allowed access to a concentrate diet because these diets allowed for higher dry matter intake and volatile fatty acid (VFA) production. Volatile fatty acids were particularly important for calves because they were required for development of rumen papillae (Suárez et al., 2007). Rumen development in calves was essential because it allows calves to eat solid feed and improved feed efficiency, which allowed for decreased feeding costs for the farmer (Berends et al., 2012b). In a 1956 study, calves eating grain, hay, and hay and grain had similar rumen growth with respect to body weight, while calves with a milk-only diet did not increase rumen weight from 0 to 16 wk of age. These data further indicated that digestion of solid feed increased rumen development in calves (Warner et al., 1956). Managing calves for maximum feed intake and the importance of housing system should not be overlooked; however, there was little data available on the effect of housing system on feed intake of dairy calves.

Feed intake could be a measure of housing system quality since calves stressed by factors such as excessive heat or illness showed decreased feed intake (Gorgulu et al., 2012; Weary et al., 2009).

The effect of housing and management on calf health

Illness in dairy calves was a source of stress, it not only negatively affected the biological function of calves, it also negatively altered the affective state (Weary et al., 2009). Pre-weaned heifer deaths accounted for the highest percentage of deaths on dairy farms and scours and digestive illness accounted for the highest percentage of these deaths at 56.5% followed by respiratory disease at 22.5% (USDA, 2007). In terms of biological function, calves experiencing infectious disease during the pre-weaning period had reduced performance when they joined the lactating herd (Svensson et al., 2003). For example, animals experiencing illness as calves had decreased reproductive performance and a higher age at first calving (Correa and Curtis, 1988).

The effects of calf housing system and management strategy on the health of dairy calves were studied extensively in recent years. Calf housing system appeared as the first significant factor for clinical cases of calf scours and pneumonia in a 1986 study assessing Holstein herds in Ontario (Waltner-Toews et al., 1986). Calves housed in outdoor hutches were significantly less likely to require treatment for scours or pneumonia than calves housed indoors in individual pens. Interestingly, calves raised indoors in group pens did not have significantly more illness than calves housed individually indoors, which was mostly attributed to decreased ventilation in indoor calf barns (Waltner-Toews et al., 1986). Calves housed outdoors in hutches were 25 times less likely to be treated for pneumonia and 8 times less likely to be treated for scours than calves raised in indoor pens again due to an increase in ventilation (Waltner-Toews et al., 1986). Increased pneumonia in indoor calf barns was especially common during winter months because barns were closed in cold weather leading to poor ventilation (Lago et al., 2006). Although calf hutches and outdoor housing for calves were recommended over indoor housing for health reasons, dairy producers continued to use calf barns because they provided better worker comfort than outdoor systems (McFarland, 1996).

One way to increase worker comfort and reduce labor associated with housing calves outdoors was by housing dairy calves in group pens (Kung et al., 1997). Higher rates of illness and mortality of group housed calves compared to individually housed calves were reported (Maatje et al., 1993). However, another recent study found no difference in calves housed in groups when compared to individually housed animals (Kung et al., 1997). Housing calves in smaller groups and providing a stable group dynamic were two management strategies that could reduce morbidity and mortality in group housed calves (Lorenz et al., 2011). Results from studies examining the health of calves housed outdoors in groups were mixed; however, there are promising results that calves housed in groups with good management did not experience higher morbidity and mortality than individually housed calves.

Another aspect of calf housing that was important for calf health was provision of bedding regardless of whether calves were housed individually or in groups, indoors or outdoors (Webster, 1984). The ideal bedding surface for calves drained well and was easy to clean. These factors positively affected calf hygiene and, therefore, calf health (Webster, 1984). There was limited research on the effect of bedding surface on calf health. Calves bedded on granite fines, sand, rice hulls, long wheat straw, and wood shavings had similar levels of IgG transfer (Panivivat et al., 2004). Calves housed on granite fines and sand had significantly more treatment days for scours than calves housed on the other surfaces. It was not known if this was an actual effect of bedding surface on health or if incidences of scours were more noticeable on the granite and sand surfaces (Panivivat et al., 2004). Calves housed outdoors with dirt flooring were found to be at significantly higher risk for infection with *Cryptosporidium* (a major cause of scours in calves; Szonyi et al., 2012). Calves were also significantly more susceptible to *Cryptosporidium* infection when housed on bedding 0 to 5 cm deep when compared to calves housed on bedding 11 to 15 cm deep (Brook et al., 2008). Bedding depth reduced the risk of *Cryptosporidium* infection because deep bedding increased calf hygiene and provided a barrier between the calf and environmental contaminates (Brook et al., 2008).

Regardless of how calves were housed, it was important to provide high quality colostrum in a timely manner. Lack of adequate colostrum was one major cause of increased illness in group housed calves or individually housed calves

(Maatje et al., 1993). Calves must ingest colostrum within the first 24 h of life (when antibodies in colostrum could best be absorbed) to receive essential antibodies against disease. The recommended minimum level of serum IgG was 1,000 mg/dL at 24 h of age to ensure optimum protection from infectious disease pathogens (Sellers, 2001). Calves with less than 1,000 mg/dl were more likely to contract illness and die than calves with adequate transfer of IgG (Sellers, 2001). In order to achieve this goal of 1,000 mg/dl of IgG, calves should be fed 3 qt of colostrum within 1 h of birth and an additional 3 qts during the next 12 h (USDA, 2007). In addition to feeding colostrum at the correct time, colostrum quality must be evaluated to ensure transfer of antibodies to the calf. Colostrum quality in beef cattle was generally very good; however, due to high milk production, colostrum quality in dairy cattle was reduced quality IgG levels were diluted (Lorenz et al., 2011b). Management of dairy calves and beef calves also differed greatly; beef calves remained with the dam and dairy calves were separated immediately and relied on human intervention to be provided with colostrum (von Keyserlingk et al., 2009). Good quality colostrum contained greater than 100 g/L of IgG (Lorenz et al., 2011). One method for checking colostrum quality, which was very cost effective for the farmer was the use of a colostrometer. Around 44% of United States dairy operations utilized a colostrometer to check if colostrum was suitable for calves (USDA, 2007). The colostrometer measured specific gravity of colostrum and was an easy on farm method for measuring immunoglobulin levels

(Fleenor and Stott, 1980). Managing calves for optimum health was related not only to housing facilities and bedding but also to colostrum management.

More research was needed to determine the effect of group housing and bedding amount and type on calf health. It was important to remember that although calf housing is important to animal health; other aspects of managements such as provision of colostrum should not be overlooked.

The effect of fly pressure on biological function

Fly pressure decreased performance in dairy calves as well as adult cattle (Estienne et al., 1991;Taylor et al., 2012). Stable fly populations exceeding 10 flies per cow led to a 40% decrease in milk yield (Bruce and Decker, 1947). There was limited information available regarding the effect of stable flies on dairy calf performance. However, data was available on the effect of stable flies on beef calves. For example, 14-mon old beef heifers exposed to stable flies gained 0.73 kg less per day than heifers kept in fly-free conditions (Campbell et al., 1977). Stable flies decreased feed efficiency in growing beef calves and led to the need for extended feeding periods to reach slaughter weight (Campbell and Berry, 1989). Although these studies were performed on beef cattle, they indicated that stable flies decreased weight gain in growing cattle which led to increased feed costs for farmers, concepts which were applicable to dairy calves. Stable flies reduced weight gain and milk production in cattle by causing pain and stress as well as energy expenditure on fly avoidance behaviors such as foot stomping and kicking (Todd, 1964). Although house flies did not bite, they negatively affected animal performance by spreading disease-causing bacteria such as *Salmonella* and *E. coli.* These organisms caused mastitis in lactating cattle when milking equipment was contaminated as well as pinkeye in adult cattle and calves. (Christensen, 1982; Stewart, 2007).

Affective state

The affective state was defined as how the animal “feels;” for example, a state of fear, pain, or stress (Fraser, 2008). Affective state was, possibly, the most difficult aspect of animal welfare to measure. It could be measured through physiology or by observation of behavior. Behaviors associated with a positive affective state in dairy calves included play behavior and exploratory behavior because these behaviors were considered non-essential to survival and did not occur when the calf was experiencing aversive circumstances such as stress caused by factors like hunger or illness (Friend and Dellmeier, 1988). Calves were highly motivated to explore their environment and did so using vision, smell, and touch (Wilt, 1985). If exploratory behavior was restricted, calves exhibited hyper-responsiveness to stimuli such as loud noises (Wilt, 1985). In turn, play behavior also indicated positive affective state in calves. Play could be observed in home pens or in an arena test (Jensen et al., 1998). Play in calves was important for developing the ability to cope with novel circumstances later in life because it provided calves practice in dealing with unexpected occurrences. Play was also considered an indicator of physical and psychological well-being because it only occurred when calves' basic needs were met (Spinka et al., 2001; Friend and Dellmeier, 1988).

Measuring affective state in calves

Behaviors associated with a negative affective state in calves included hyper-responsiveness and stereotypies (Wilt, 1985). Calves exhibiting hyper-responsiveness to stimuli such as loud noises needed increased stimuli to explore. Calves housed in restrictive stalls as opposed to group pens startled more easily and exhibited hyper responsiveness to stimuli such as a person entering the barn (Wilt, 1985). Stereotypic behavior was defined as being: "unvarying and repetitive with no apparent function" (Mason and Rushen, 2008). Stereotypies observed in dairy calves included tongue rolling and bar biting. These behaviors were thought to be due to frustration associated with lack of natural grazing behavior in adult cattle and occurred because of an unfulfilled desire to perform suckling behavior in calves (Mason and Rushen, 2008;De Passillé et al., 2010). Using observational techniques to focus on play behavior and stereotypic behavior made it possible for farmers and researchers to quickly and inexpensively evaluate the affective state of calves (Mintline et al., 2013; Mason and Rushen, 2008). Affective state could also be evaluated in a more quantitative manner using physiological measurements (Adcock et al., 2006).

The affective state of calves was typically evaluated physiologically by measuring levels of stress hormones, generally cortisol (Doherty et al., 2007). Cortisol was released from the adrenal cortex in response to signals from the hypothalamus and anterior pituitary and this process was initiated when the animal as faced with an acute or chronic stressor. Cortisol was a steroid hormone, which bound to receptors in the cytoplasm then traveled to the cell nucleus and influenced the production of messenger RNA (McEwen, 1998). A major role of cortisol was to alter metabolism to increase blood glucose (Dickerson and Kemeny, 2004). Cortisol increased blood glucose levels by increasing the synthesis of glucose by liver cells and by stimulating breakdown of protein and fat stores in tissue (Dickerson and Kemeny, 2004). In addition to its effects on metabolism, cortisol also influenced immunity by decreasing lymphocyte numbers and production of pro-inflammatory cytokines (Weston et al., 1973). These responses caused by cortisol release allowed the animal to respond to a stressor and return to homeostasis (McEwen, 1998).

There were two types of stress commonly encountered by animals. The first was acute stress, which occurred when a stressor was applied and the stress response was shut off once the stressor was removed, allowing the animal to enter a period of recovery. Acute stress was considered to be an adaptive response because it assisted the animals' body in dealing with the stressor and returning to homeostasis through actions such as increased availability of glucose (Trevisi and Bertoni, 2009). A second type of stress experienced by animals was chronic stress, which involved application of repeated acute stressors or multiple acute stressors. The chronic response was characterized as a stress response which lacked a period of recovery (Trevisi and Bertoni, 2009).

The chronic stress response was considered maladaptive and could lead to side effects such as decreased immunity which negatively impacted animal welfare (Trevisi and Bertoni, 2009).

When a stressor is acute and the animal enters a period of recovery this was defined as eustress because it is beneficial to the animal (Selye, 1976). Examples of responses associated with acute stress (or eustress) were increased heart rate and blood pressure and decreased gastrointestinal motility which were in place to allow the animal to mount a fight or flight response in reaction to the stressor (Wielebnowski, 2003). This response was beneficial because the increase in stress hormone levels improved the animals' ability to respond to the stressful stimuli and return to homeostasis. When an acute stressor was repeated or there were multiple acute stressors at once the animal shifted to a maladaptive state known as distress (Breazile, 1987). Distress occurred when the animal was unable to enter a period of recovery and was subjected to prolonged high levels of stress hormones. This led to decreased immunity and an increased susceptibility to disease, which were detrimental to animal welfare and production (Clark et al., 1997).

Plasma total cortisol analysis was commonly used to evaluate cortisol levels that were stress-related. This method measured: free cortisol, that which was loosely bound to albumin, and cortisol tightly bound to Corticosteriod Binding Globulin (CBG; Adcock et al., 2006). Total cortisol analysis measured forms of cortisol which were biologically available to the cell (free cortisol and cortisol loosely bound to albumin) as well as cortisol tightly bound to CBG, which was not available to cells. In order to determine the amount of active cortisol available to the cell, the free cortisol index (FCI) should be calculated. This was done by measuring total plasma cortisol concentration divided by plasma CBG concentration (Roux et al., 2003). Regardless of the method used to evaluate affective state in dairy calves, the importance of the influence of management strategy and housing type on affective state should not be overlooked.

The effect of feeding management on affective state

As previously mentioned, management could negatively or positively influence affective state of calves. Hunger was a contribution to negative affective state in dairy calves. Examples of behaviors in dairy calves associated with hunger included non-nutritive suckling of pen fixtures and even other calves (cross-suckling) as well as head butting pen fixtures and feeding equipment (Herskin et al., 2010). Dairy calves were often managed in what was considered an “unnatural” environment. In a natural setting, calves stayed close to the dam for around 2 wk and suckled 4-10 small milk meals per day (Fraser and Weary, 2004; Webster, 1984). In contrast, on most dairy farms, calves were removed from the dam at birth to reduce disease transmission and fed milk twice per day. This infrequent feeding schedule led to decreased intake and hunger for the calf (Herskin et al., 2010). Although it was not feasible to leave the calf with the cow on most dairy farms, one option to reduce hunger and improve intake in dairy calves was by providing smaller, more frequent meals via a teat instead of a bucket (Jensen, 2003). Feeding calves multiple times per day could be labor intensive; however, this could be reduced by feeding calves in groups utilizing a mob feeder with artificial teats or a computerized calf feeding system (Kung et al., 1997; Hepola, 2003). These computerized feeding systems worked best for calves housed in groups, which not only addressed the concern of hunger but also reduced isolation from conspecifics (Hepola, 2003).

The effect of isolation on affective state

Isolation from the cow and other calves was a second major concern that resulted in a negative affective state in dairy calves. Calves were removed from the cow promptly after birth and isolated from other calves to reduce the risk of illness (Fraser, 2008). Recently, group housing of calves has become more popular, and there was promising research stating that, with good management, dairy calves could be housed in groups with a low risk of illness (Lorenz et al., 2011a). Housing calves in groups positively influenced their affective state, because calves were social animals that could derive comfort from each other (Friend and Dellmeier, 1988). Calves housed in groups also participated in group play behavior, which was an indicator of psychological and physical well-being (Friend and Dellmeier, 1988). There was legislation in the UK (Statutory Management Regulation 16) that required calves over 8 wk old to be housed in group pens (Department for environment, food & rural affairs, 2012). This legislation was enacted because of the negative effects of housing calves in small individual stalls. The aim of providing a group pen requirement for veal calves was to increase animal welfare by increasing natural living through social interactions and higher space allowance (Xiccato et al., 2002). In addition to reducing stress, housing veal calves in groups was economically advantageous for producers (Andrighetto et al., 1999).

The effect of space allowance on affective state

In addition to isolation, space restriction was a common aspect of management that negatively altered the affective state of dairy calves. Restricted space allowance was considered one of the most common challenges to raising calves in confinement (Friend and Dellmeier, 1988). Recommendations stated that calves needed at least 1.5 m^2 of space per animal (Chua et al., 2002). Inadequate space was of particular concern in dairy calves raised for veal. Traditionally veal calves were housed in crates until slaughter, making them ideal subjects for studying the effects of limited space allowance on dairy calves (Wilt, 1985). Confining calves to small crates or pens led to several qualitative signs of negative affective state. Calves housed with limited space to move had impaired locomotion, such as stumbling, when they were allowed access to an arena, this may be due to deprivation to practice locomotor behaviors (Dellmeier et al., 1985). Furthermore, calves housed with limited space also showed stereotypic behaviors such as chewing pen fixtures due to lack of adequate stimulation (Dellmeier et al., 1985). Veal calves in restrictive housing showed increasing discomfort as they grew, which was evident during repeated attempts to stretch the hind legs as well as the adoption of abnormal lying postures (Wilt, 1985). The effect of restrictive housing could also be shown quantitatively. Calves housed in restrictive crates had the highest cortisol concentrations, indicating negative affective state, when compared to calves housed in pens, hutches, or group housing (Friend and Dellmeier, 1988). Due to increased public perception of issues associated with veal calf housing, new laws were in place to change methods of housing calves, especially in European countries (Xiccato et al., 2002).

Veal calves reared in groups with higher space allowance had higher final live weights than calves housed individually. In addition, group housed veal calves had higher feed efficiency than individually housed calves (Xiccato et al., 2002). Rearing veal calves in groups also helped improve public perception of the veal industry, thereby making veal more marketable (Xiccato et al., 2002). These results were not only applicable to veal calves, as calves being raised in groups as dairy replacements showed similar advantages (Kung et al., 1997). In addition to concern about the effect of space allowance on affective state, the influence of painful management practices such as dehorning on the affective state of calves should not be ignored.

The effect of painful procedures on affective state

Dehorning or disbudding dairy calves was essential to protect dairy workers as well as other animals from possibly dangerous horns (Stull and Reynolds, 2008). In the United States the disbudding procedure (removal of horn tissue before it attaches to the periosteum) was usually performed when calves were around 2 months old. This process could be performed with a hot iron, caustic paste, or a specialized scoop (Stull and Reynolds, 2008). Although beef cattle producers have introduced polled genetics, leading to a 58% reduction in horned beef calves from 1992 to 2007, this trend was not evident in the dairy industry, where 94% of operations dehorn calves (USDA Dairy Survey, 2007). Regardless of the type of disbudding performed, calves displayed behavioral and physiological signs of negative affective state during and after disbudding (Stock et al., 2013). Calves displayed behavioral signs of pain, including head shaking and rearing during and after disbudding with a hot iron. These calves also had elevated plasma cortisol and ACTH levels, which were physiological indicators of pain or stress (Graf and Senn, 1999). Disbudding with caustic paste caused similar effects including behavioral signs such as head shaking, ear flicking, and head rubbing for 1 h after application of paste with the most severe effects occurring 30 minutes after paste application (Braz et al., 2012). Due to its influence on the affective state of dairy calves, disbudding was a source of negative public perception of the dairy industry; this produced negative financial effects for farmers (Stock et al., 2013). Change in management strategies including the use of anesthetics, such as lidocaine, prior to dehorning and analgesics, such as tramadol, after dehorning reduced physiological and behavioral signs of pain and distress in calves (Mintline et al., 2013; Braz et al., 2012). Countries in the European Union, as well as Australia, had specialized legislation governing which types of dehorning were appropriate, when calves should be dehorned, and if pain relief or anesthetics were required (Stock et al., 2013). In the United Kingdom dehorning with caustic paste was allowed in calves > 1 wk of age, however the Protection of Animals Act 1954/1964 required that local anesthesia be used when dehorning using cautery or amputation (Kent, 1999). In Australia it was recommended that dehorning without anesthetic not be allowed in calves under 6 months old (Model Code of Practice for the Welfare of Animals: Cattle, 2004). On the other hand, dehorning was not legislated in the United States and pain relief during dehorning was not commonly used. However, this may change in the future due to pressure from the public (Stock et al., 2013).

The effect of heat or cold stress on affective state

In addition to limited space allowance, another environmental factor that may influence the affective state of dairy calves was exposure to hot or cold environments when calves are housed outdoors (Webster, 1984). Heat stress in dairy calves occurred at temperatures above 32° C and was marked by increased body temperature as well as increased heart rate (Neuwirth et al., 1979). Cold stress in calves occurred between 6° and 18° C. Environmental temperature was an example of a factor affecting several aspects of animal welfare. Housing calves in hot or cold conditions not only influenced their affective state, but also altered natural behaviors and influenced performance (Friend and Dellmeier, 1988). For example, housing calves in an environment with a high ambient temperature could alter lying behavior (natural living) by increasing time spent lying laterally (Webster, 1984). Housing calves in a cold environment could lead to decreased transfer of immunoglobulins through colostrum which led to increased illness, a factor in calf performance as well as affective state (Olson et al., 1980). Heat stress was considered one of the biggest challenges to production in lactating cattle in the southeast (West, 2003). High temperatures and high relative humidity not only reduced production, but also caused discomfort and stress to adult dairy cattle (West, 2003). Heat stress also caused increased plasma cortisol concentrations and decreased innate immune function in adult cattle (Carroll et al., 2013). It is likely that heat stress occurred in calves as well as adult cattle, especially since neonatal calves could not thermoregulate as efficiently as adult animals (Hill et al., 2011). The effects of heat stress on dairy calves were not studied in as much depth as the effects on adult cattle, but some data were available. Heat stress in dairy calves caused decreased ADG, which led to higher calf feeding costs (Hill et al., 2011). Calves housed in a well-ventilated area with shade had greater ADG, lower incidence of scours, and lower respiration rate than animals housed without shade indicating shade and ventilation improved both performance and affective state of dairy calves housed in warm environment (Hill et al., 2011). In addition to shade and ventilation, the use of inorganic bedding lowered the skin surface temperature of calves which reduced heat stress (Sutherland et al., 2013).

Calves exposed to cold weather displayed behavioral changes indicative of negative affective state, such as shivering, posture changes, and shelter seeking (Fisher, 2007). Calves also had physiological changes, such as vasoconstriction and increased stress hormone levels (Godfrey et al., 1991). Cold stress led to decreased immunity possibly caused by increased levels of stress hormone; this in turn led to increased incidence of diarrhea or respiratory diseases which negatively influenced affective state as well as calf performance (Frank et al., 2003; Nonnecke et al., 2009). The effect of cold weather on calf immunity may be profound, in a study of 438 calves the morbidity rate (a factor closely associated with affective state) for calves born during winter was 52%, while morbidity rate was only 13% in calves born during the summer months, this suggested not only a welfare concern but an economic concern for farmers as well (Godden et al., 2005). It was important to note that the effects of cold stress including increased plasma cortisol concentration were more severe in calves with thin hair coats or small body size (such as Jerseys) or those adapted for warm climates (such as Brahmans; Holmes and McLean, 1975; Godfrey et al., 1991).

There were several ways to reduce the effects of cold stress on pre-weaned dairy calves with the provision of clean, dry bedding one of the most important. The "critical temperature" was defined as the environmental temperature at which an animal must expend energy to maintain its internal body temperature (Webster, 1984b). The lower critical temperature for calves lying on concrete was 18 ° C, while the lower critical temperature for calves provided dry straw was 6 ° C (Webster, 1984a). This indicated the provision of dry bedding was important in helping calves cope with cold stress. Dry bedding reduced heat loss due to conduction of heat from calves to cold flooring (Webster, 1984a). Certain types of bedding, such as straw, allowed calves to “nest” which trapped heat in cold conditions. However, if bedding was wet, calves were unable to practice nesting behavior (Webster, 1984a). Steers housed during winter in South Dakota gained more weight when provided bedding compared to those house on concrete during the coldest periods of the study, indicating that bedding was necessary for optimum weight gain in cold conditions (Anderson et al., 2005). In addition to providing bedding, calves should be provided with shelter from wet and windy conditions (Fisher, 2007). Heifers provided shelter had a higher rate of gain than those exposed to wet and windy conditions, sheltered animals also did not show postural changes and shivering like exposed heifers (Holmes and McLean, 1975).

The effect of fly pressure on affective state

It was clear from research performed on calves housed in hot and cold environments that adjustments to housing system and bedding material were necessary as seasons change. Adjustment to bedding material was particularly important during the summer months because fly pressure increased (Schmidtmann, 1991). Stable flies fed on blood by biting the lower legs of cattle causing stress and pain indicated by increased plasma cortisol concentrations and aversive behaviors such as kicking and stomping in an attempt to dislodge flies (Estienne et al., 1991; Doherty et al., 2007). An additional behavioral indication of stress by stable flies was that cattle affected by 15 flies/animal made no attempt to lie down (Todd, 1964). This response was indicative of stress caused by flies because there was a significant reduction in a behavior which cows prioritize very highly, as mentioned previously, cows chose to lie down over eating when deprived of both behaviors (Munksgaard et al., 2005). In addition to causing behavioral indicators of negative affective state, flies influenced physiological indications of stress as well. When cattle were exposed to 25-50 stable flies/animal for 1 h there was a significant increase in serum cortisol levels, indicating a physiological stress response caused by fly feeding (Schwinghammer et al., 1987).

Conclusions

The ways in which housing and management affected animal welfare have been closely studied in lactating dairy cattle. Lying behavior was essential in increasing milk production through increased mammary blood flow, and stall comfort was one of the most important non-nutritional causes of variation in milk production (Haley et al., 2000; Bach et al., 2008) Promising research was being conducted on the effect of housing and management on the welfare of dairy calves. Soft, dry bedding was shown to increase lying time in dairy calves similar to adult cattle (Camiloti et al., 2012). Bedding was an important aspect of housing management and housing environments which allowed for natural living and behavior to increased production (biological function) in both adult cattle and dairy calves; for example, lying behavior has been shown to be associated with weight gain in heifers (Mogensen et al., 1997). The affective state of dairy calves, especially veal calves, has also come under close scrutiny in recent years. The effect of thermal stress seemed to be similar in adult cattle and dairy calves (Holmes et al., 1978). Fly pressure could negatively influence affective state in adult cattle and calves (Eicher et al., 2001; Campbell et al., 1977).

Despite extensive knowledge of housing and management requirements of adult dairy cattle, further research was still needed on best housing and management practices for dairy calves, especially as calf welfare became more of a public concern and legislation in European countries was in place to regulate farm procedures (such as the use of lidocaine for dehorning) and housing systems (such as requirements for group housing; von Keyserlingk et al., 2009). It was possible that this legislation may reach the United States in the future, where it will be beneficial to have a good knowledge base surrounding dairy calf welfare.

The overall objective of this project was to determine the effect of two aspects of calf management 1) bedding type and 2) fly pressure on the natural living/behavior, biological function, and affective state of pre-weaned Holstein and Jersey dairy calves. These management factors are closely inter-related because bedding type has a significant effect on fly development, in fact, inorganic bedding materials can reduce stable fly and house fly larvae numbers by up to 99% (Schmidtmann, 1988). This objective was selected due to increasing popularity due to decreased production of flies and easy management of inorganic bedding materials such as gravel in Tennessee. The current study examined the effects of these bedding materials during cooler months than many previous studies and also included the effect of Holstein vs. Jersey breed, which was not evaluated previously. The objective of evaluating the effect of fly pressure addressed the limited data on the effect of flies on dairy calves, although there is some current research on the effect of flies on beef calves.

It was hypothesized that sand or rubber mats would increase lying time and decrease lying bouts due to providing a soft surface, but not affect body weight gain or feed intake in pre-weaned Holstein and Jersey calves due to relatively mild fall and winter temperatures being previously recorded in Tennessee. It was also hypothesized that as stable and house fly populations increased, calf welfare would decrease. This hypothesis was based on studies currently available, which demonstrated that flies decreased essential behaviors, such as lying behavior, and decreased performance by decreasing weight gain and feed efficiency (Todd, 1964; Campbell et al., 1977).

CHAPTER I
THE EFFECT OF BEDDING SURFACE ON THE WELFARE OF PRE WEANED HOLSTEIN AND JERSEY CALVES

Abstract

Research on the effect of bedding surface on the welfare of dairy calves has produced mixed results with some studies indicating that calves prefer a soft, dry surface and others suggesting that bedding or flooring have no effect on behavior and performance. The objective of this study was to determine the effect of bedding on the behavior and performance of Holstein and Jersey calves housed using individual hutches bedded with gravel, rubber mats, or sand. It was hypothesized that sand or rubber mat would increase lying time and decrease lying bouts, but not affect BW gain or feed intake in pre-weaned calves. Twenty-three Holstein calves and 38 Jersey calves were blocked by birth date and randomly assigned to 1 of 3 bedding treatments (gravel, rubber mat, or sand). Data were collected for 6.5 to 10 wk following birth depending on when calves were weaned. Lying time and lying bouts were assessed with dataloggers, recording at 1-min intervals for 6 consecutive d each wk. Biological function was determined by weight gain (calculated from birth weight and weekly BW) and grain intake (calculated as daily difference between grain offered and refused over 3-d). Plasma cortisol levels were determined using a commercially available RIA kit. All animal procedures were approved by the University of Tennessee IACUC committee (protocol #2117-0712). Data were analyzed using a mixed model in SAS with repeated measures. There was a significant effect of breed for all response variables. Mean lying time was not affected by trt, wk, or trt × wk interactions for Holstein and Jersey calves. Lying bouts were unaffected by trt, wk, or trt × wk interactions for Holstein calves. Lying bouts were affected by trt and trt × wk interactions, but not by wk for Jerseys. Jersey calves on mats engaged in more lying bouts/d than calves housed on gravel or sand. Body weight of Holstein and Jersey calves increased over time, but no effects of trt or trt × wk. An effect of week was evident in BW gain for Holstein and Jersey calves but trt or trt × wk interaction effects were not evident. Grain intake increased as the study progressed for Holstein and Jersey calves, but no treatment effect was evident. There was no effect of trt or trt × wk on plasma cortisol levels in Holstein calves, however there was an effect of wk. These data indicate that there were no biologically significant differences in behavior, performance or physiology among treatments. This suggests that, on a well-managed farm, any of these beddings may be used without compromising the welfare of pre-weaned Holstein and Jersey calves.

Introduction

Animal welfare can be defined by 3 parameters: natural living and behavior, biological function, and affective state (Fraser, 2008). If any one of these areas is deficient the welfare of the animal can be considered to be lacking (Fraser, 2008). Deficient animal welfare can cause decreased animal production indicated by factors such as decreased feed intake or increased incidence of disease caused by stress which can lower weight gain (Neindre, 1993). Animal welfare is particularly important when housing animals in intensive production systems, which differ from their natural environment (Dellmeier et al., 1985). Around 75% of dairy calves in the United States were housed individually in commercial hutches during the pre-weaning period to reduce disease transmission (USDA, 2007). The ideal bedding surface for calves in hutches was non-slippery, well drained, soft, and easy to keep clean (Webster, 1984a). Bedding within the hutch was crucial to maintaining animal comfort in intensively managed dairy calves indicated by the fact that provision of bedding increased essential behaviors such as lying and increased indicators of production such as weight gain (Camiloti et al., 2012; Anderson et al., 2005).

Dairy calves spent up to 70-80% of their daily time budget lying down (Chua et al., 2002). Lying behavior was an important indicator of animal comfort and welfare in adult dairy cattle and calves indicated by the fact that lying behavior took up a large portion of the time budget of adult cattle and calves as well as the fact that lying behavior can increase production parameters (Ito et al., 2009; Chua et al., 2002; Haley et al., 2000; Mogensen et al., 1997). Adequate lying time (12-14 h/d) increased milk production and reduced stress in adult cattle and may have increased weight gain in dairy heifers (Haley et al., 2000; Mogensen et al., 1997). One aspect of dairy animal management that might increase lying time in cattle was providing a soft, dry bedding surface. When given a choice between sawdust with various levels of moisture and bare concrete, calves chose to lie down on the driest surface available and always avoided bare concrete because concrete provided a hard surface (Camiloti et al., 2012). Collectively these studies suggested that calves preferred bedding that provided a soft, dry surface.

It is possible that bedding surface may affect biological function of calves. Biological function goals for dairy calves include weight gain and increased grain consumption. In order to be bred as early as possible (around 15 months of age), calves should be managed to maximize weight gain (Davis Rincker et al., 2011). Maximizing growth in pre-weaned calves also affected future milk production (Van Amburgh, 2008). Since providing bedding may increase lying behavior, which in turn can influence weight gain, the addition of bedding may allow for improved weight gain in dairy calves. When weaned calves were given access to either a bare floor or a bedded floor during winter in North Dakota, calves with access to bedding had greater weight gain during the coldest months of the year (Anderson et al., 2005). In contrast calves housed on several types of organic and inorganic surfaces (gravel, sand, rice hulls, straw, and wood shavings) in a study taking place from August-October in Arkansas showed no difference in weight gain (Panivivat et al., 2004). Calves housed on a variety of inorganic surfaces (slatted floors, rubber mats, and concrete) did not differ in weight gain or feed efficiency (the timeline of this study was not specified; Yanar et al., 2010).

The affective state of calves was typically quantified in animals by measuring concentrations of the stress hormone, cortisol (Doherty et al., 2007). Chronic stress may occur when calves were subjected to a stressor, such as cold weather or an uncomfortable environment for an extended period of time (Trevisi and Bertoni, 2009). Chronic stress caused a continuous increase in stress hormones and may have negatively affected immunity and metabolism (Trevisi and Bertoni, 2009). Plasma total cortisol analysis was commonly used to evaluate cortisol levels related to stress. This method measured free cortisol, cortisol loosely bound to albumin (both were available to the cell), and cortisol tightly bound to Corticosteriod Binding Globulin (CBG; unavailable to the cell; Adcock et al., 2006). In order to determine the amount of active cortisol available to the cell the free cortisol index (FCI) must be calculated. This was done by measuring total plasma cortisol concentration divided by plasma CBG concentration (Roux et al., 2003).

Although there were several studies about the effect of bedding surface on dairy calf welfare, most of these have been performed in warmer months. Our study was unique in that calves were enrolled from September - March. Most of the current literature on calf bedding surrounded the use of organic materials for bedding with our focus being three inorganic bedding options. Finally, little was known about the effect of bedding on the affective state of the animal and we attempted to address this question by including plasma cortisol analysis in our response variables. Because of these deficits in the current literature our study was designed to include inorganic bedding, both Jersey and Holstein calves as well as plasma cortisol analysis.

The objective of this study was to determine the effect on the behavior, performance, and affective state of Holstein and Jersey calves housed using individual hutches bedded with gravel, rubber mats, or sand.

Materials and Methods

Animals, housing, and management

Twenty-three Holstein and 38 Jersey calves born from September 2012 to March 2013 were enrolled on this study. All calves received 4 L of colostrum within 12 h of birth and were housed in commercial plastic calf hutches (Calf-Tel, Hampel Animal Care, Germantown, WI) with an outside wire enclosure and racks to hold buckets for starter grain and water. Calf starter utilized at both farms contained 18% protein and 2% crude fat (tag values, Tennessee Farmers Co-op, LaVergne, TN). All calves were fed 4 L waste milk once daily and water was provided *ad libitum*. Calves were blocked by birth date and randomly assigned to 1 of 3 hutch bedding treatments: gravel (typically 2.5 cm in diameter), rubber mat (1.3 cm thick) over a gravel base, or river sand (5 cm deep) over a gravel base (gravel used for base was typically 2.5 cm in diameter). Calf hutches measured 2.1 m × 1.2 m inside and had an attached outside area measuring 1.1 m × 1.7 m surrounded by a 1.3 m high wire fence. All animal procedures were approved by the University of Tennessee IACUC committee.

Holstein calves (gravel n = 8, mat n = 7, sand n = 8) were housed at the Middle Tennessee Research and Education center (MTREC) in Spring Hill, TN. Calf hutches at MTREC were placed on a gravel pad measuring approximately 12 m × 54 m**.** Holstein calves were offered 0.21 ± 0.02 kg calf starter initially and this was increased in 0.2 kg increments as needed to ensure *ad libitum* access. Holstein calves were weaned at 6.5 ± 0.2 wk of age.

Jersey calves (gravel n = 14, mat n = 11, sand n = 13) were housed at the Dairy Research and Education Center (DREC) in Lewisburg, TN. The DREC calf hutches were placed on a gravel pad measuring approximately 21 m × 61 m. At 6 wk of age, milk for Jersey calves was reduced to 2 L once/d. All Jersey calves were offered 0.2 kg calf starter initially and this was increased in 0.2 kg increments as needed to ensure *ad libitum* access. All Jersey calves were weaned at 10 wk of age.

Lying behavior

Mean lying time (min/d) and lying bouts (n/d) of all calves were determined using the HOBO pendant G accelerometer data logger (Onset Corporation, Bourne, MA; Bonk et al., 2013). Loggers were attached to the lateral side of calves' hind legs above the pastern joint with vet wrap. Calves were fitted with a logger after birth during the move to individual hutches. Loggers were removed and replaced each week on alternating legs to prevent skin irritation. Loggers recorded posture at 1-min intervals for 6 d/wk throughout the study. Lying time and number of lying bouts were calculated as described by (Bonk et al., 2013).

Performance measurements

On the day of birth, all calves were weighed and time of birth and time of first colostrum were recorded. Calving ease was also recorded on a scale of 1-5 with 1 being no problems and 5 being extremely difficult birth (Cappel et al., 1998). All calves were removed from the hutch each week to be weighed until weaning to determine body weight gain using a mobile scale (Tru-Test, Tru-Test

Limited, Auckland, NZ). Calf starter grain intake from all calves was determined for 3 d each week by daily grain offered (Monday, Tuesday, and Wednesday) and refused (Tuesday, Wednesday, Thursday). Calf health was evaluated by farm staff at each location according to the University of Wisconsin calf health scoring chart guidelines (Lago et al., 2006). Type and duration of any treatment administered to calves was recorded.

Cortisol and CBG

Blood samples were collected from all calves on the day of birth and once weekly until weaning. Approximately 10 mL of blood was collected via jugular venipuncture into a heparinized vacutainer tube (BD vacutainer, Franklin Lakes, NJ). Blood samples from Jersey calves were taken with calves in hutches, while Holstein calves were moved approximately 1.5 m from the housing area to a mobile head catch for blood sampling. Care was taken to obtain a sample in 2 min or less to decrease calf stress and the possibility of elevated plasma cortisol concentrations. Vacutainer tubes were spun at 3,000 × *g* for 10 min immediately after sampling was completed. Separated plasma was aliquoted into two 2 mL cryogenic vials with a disposable transfer pipette and stored at -20 °C until analysis. Plasma total cortisol concentrations were determined on duplicate samples using an RIA procedure (Coat-A-Count, Diagnostic Products, Los Angeles, CA) and were counted for 1 min using a gamma counter (Wizard Automatic Gamma Counter, PerkinElmer, Waltham, MA). Plasma cortisol concentrations < 3 ng/mL were considered to be below detectable limits. Intraand inter-assay CV was determined for low (10 ng/mL), medium (30 ng/mL), and high (50 ng/mL) bovine cortisol standards. Intra-assay CVs were 10.7%, 5.6%, and 6.6% for low, medium, and high standards respectively. Inter-assay CVs were 12.3%, 9.6%, and 7.1% for low, medium, and high standards respectively. Plasma bovine corticosteroid-binding globulin (CBG) was measured in triplicate for all Jersey calves to determine change over time by direct ELISA as described by Roberts et al. (2003). Free cortisol index (FCI) was calculated using the total cortisol/CBG ratio.

Statistical analysis

The experimental unit was the individual calf. The effects of bedding type (gravel, mat, or sand) and breed (Holstein or Jersey) were determined using a mixed model ANOVA with repeated measures in SAS (SAS institute, Cary, NC). The model used was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \Gamma_k + \gamma_l + e_{ijkl}$$

Where μ is the overall mean, α_i is the fixed effect of treatment, β_j is the fixed effect of week, Γ_k is the fixed effect of breed, γ_l is the random effect of calf (treatment), and e_{ijkl} is the residual error term. Bedding treatments were compared using least square means with Tukey's adjustment. Differences between means were declared significant at $P < 0.05$ and trends were declared at $P < 0.09$. There was an effect of breed ($P < 0.001$) for all variables so data from the two breeds were analyzed separately. Cortisol data were log transformed, log transformed means and backtransformed means are presented.

RESULTS

Two Holstein calves housed at MTREC were removed for medical reasons (1 euthanized after going down, 1 died due to bloat), 4 Jersey calves housed at DREC were removed for medical reasons (4 died due to scours) and 1 Jersey calf was removed for non-medical reasons (stolen). There was an average low temperature of 0 ± 0.5 ° C and an average high temperature of 16 ± 0.6° C at MTREC (Holsteins) and an average low temperature of 0 ± 0.4 °C and an average high temperature of 15 ± 0.4 °C at DREC (Jerseys) over the 30 wk study period. Table 1 includes the costs including hauling of each bedding material and can be found in the appendix.

Lying time

Holstein calves spent more time lying (979.9 ± 13.5 min/d) than Jersey calves (917.5 ± 10.8 min/d lying; $P < 0.001$). Mean lying time for Holstein calves (968.9 ± 35.5 min/d; figure 1a; all tables and figures are placed in the appendix) was not affected by trt ($P = 0.73$), wk ($P = 0.44$), or trt × wk interactions ($P = 0.89$). Mean lying time for Jersey calves (916.2 ± 16.0 min/d; Figure 1b) was also not affected by trt ($P = 0.95$) or wk ($P = 0.31$). However, there was a trend for an effect of trt × wk ($P = 0.08$).

Lying bouts

Holstein calves had a higher number of lying bouts/d (13.1 ± 0.4 bouts/d) than Jerseys (10.3 ± 0.3 bouts/d; $P < 0.001$). Mean lying bouts for Holstein calves (figure 2a) was not affected by trt ($P = 0.11$), wk ($P = 0.14$) or trt × wk ($P = 0.47$). Mean lying bouts for Jersey calves (figure 2b) housed in hutches with rubber mats (11.6 ± 0.6 bouts/d) were higher (*P* = 0.01) than for calves housed on gravel (10.0 ± 0.6 bouts/d) or sand (9.2 ± 0.6 bouts/d). There was a tendency for an effect of wk (*P* = 0.08) and no effect of trt × wk (*P* = 0.17).

Body weight and body weight gain

Mean body weight of Holstein calves (53.3 ± 0.6 kg) was greater than mean body weight of Jerseys (30.4 ± 0.6 kg; *P* < 0.001). Mean body weight of Holstein calves (52.7 ± 9.3 kg; figure 3a) was not affected by trt (*P* = 0.75) or trt × wk (*P* = 0.92); however, there was an effect of wk (*P* < 0.001) with calves' weight increasing from 40.6 ± 1.2 kg in wk 1 to 65.5 ± 1.7 kg in wk 7. Mean body weight of Jersey calves (30.5 ± 0.9 kg; figure 3b) was also not affected by trt (*P* = 0.87) or trt × wk (*P* = 0.26); however, there was an effect of wk (*P* < 0.001) with calves' weight increasing from 23.3 ± 0.6 kg in wk 1 to 39.7 ± 0.6 kg in wk 8. Holstein calves had higher weight gain (4.3 ± 0.2 kg/wk) than Jersey calves (2.2 ± 0.1 kg/wk; *P* < 0.001). Mean body weight gain (4.1 ± 1.5 kg/wk) (figure 4a) of Holstein calves was not affected by trt (*P* = 0.68) or trt × wk (*P* = 0.15). However, there was an effect of wk (*P* = 0.006). Holstein calves gained an average of 3.8 ± 0.9 kg/wk during wk 1 which increased to a maximum of 6.0 ± 1.7 kg/wk during wk 5 before decreasing to 2.7 ± 1.5 kg/wk during wk 7. Mean body weight gain (2.2 ± 0.2 kg/wk; figure 4b) of Jersey calves was not affected by trt (*P* = 0.18) but there was an effect of wk (*P* < 0.001) and trt × wk (*P* = 0.04). Jersey calves gained 1.1 ± 0.3 kg/wk during wk 1, which increased to a maximum of 3.6 ± 0.3 during wk 6.

Grain intake

Holstein calves consumed more grain (771.6 ± 32.5 g/d) than Jersey calves (446.9 ± 24.8 g/d; $P < 0.001$). Mean grain intake (603.4 ± 378.8 g/d; figure 5a) of Holstein calves was not affected by trt ($P = 0.71$) or trt × wk ($P = 0.53$); however, there was an effect of wk ($P < 0.001$). Holstein calves had a mean grain intake of 426.4 ± 54.2 g/d during wk 1 which increased to a maximum of 1218.0 ± 87.2 g/d during wk 6. Intake data from wk 7 was not included in the final analysis due to storms with high winds, which caused grain to spill. Mean grain intake (446.4 ± 44.6 g/d; figure 5b) of Jersey calves was not affected by trt ($P = 0.60$) or trt × wk ($P = 0.19$) however there was an effect of wk ($P < 0.001$). Jersey calves consumed no grain during wk 1 because grain was not offered until calves were 7 d old. Jersey calves consumed a maximum of 1105.6 ± 34.8 g/d during wk 8. One calf was removed from the final analysis due to repeatedly spilling grain.

Cortisol and CBG

There was an effect of breed ($P < 0.001$) for cortisol levels. Holstein calves had a mean plasma cortisol concentration of 33.5 ± 6.8 nmol/L and Jersey calves had a mean plasma cortisol concentration of 43.4 ± 6.1 nmol/L (backtransformed means). Mean cortisol levels (log transformed means; figure 6a) of Holstein calves were not affected by trt ($P = 0.61$) or trt × wk ($P = 0.75$). However, there was an effect of wk ($P < 0.001$). Plasma cortisol levels decreased from wk 1 to wk 7 before increasing slightly in wk 8. Mean cortisol levels (log transformed means; figure 6b) of Jersey calves were not affected by trt ($P = 0.51$) or trt × wk ($P = 0.92$). However, there was an effect of wk ($P < 0.001$). Plasma cortisol levels decreased from wk 1 to wk 7 before increasing in wk 8, similar to the pattern observed in Holstein calves.

CBG and FCI were analyzed in Jersey calves for week effect only. There was an effect of week for plasma CBG levels ($P < 0.001$; figure 7a). Plasma CBG levels decreased from 1.2 ± 0.1 ng/µL during wk 1 to 0.9 ± 0.1 ng/µL during wk 6 before increasing to 1.6 ± 0.1 ng/µL during wk 10 (backtransformed mean). There was an effect of week for FCI ($P < 0.001$; figure 7b). Plasma FCI (cortisol/CBG) was at a maximum during wk 1 of 242.9 ± 21.9 nmol/L and decreased to a minimum of 7.2 ± 21.9 nmol/L during wk 7 after week 7 FCI increased to 24.5 ± 22.2 nmol/L by wk 10 (backtransformed means).

DISCUSSION

The only significant difference observed in behavior of calves housed on gravel, rubber mats, and sand was an increased amount of lying bouts in Jersey calves housed on mats possibly due to discomfort associated with wet skin and heat loss. Based on visual observation of calves, those housed on rubber mats were wetter and dirtier than those housed on gravel or sand. This effect was likely only observed in Jersey calves because they are smaller and therefore have more surface area for heat loss by conduction with cold, wet flooring. Smaller bodied animals have greater surface area because they have a larger surface area to volume ratio, which means that heat is lost quickly because there is a large area to lose heat but a relatively small volume to retain heat. There were no differences in weight, weight gain, or grain intake associated with bedding treatment. There was also no difference in cortisol levels associated with bedding treatment. These results indicated that gravel, rubber mats, or sand may all be used as bedding materials for pre-weaned dairy calves without decreasing performance or causing increased levels of stress.

Lying time

In the literature, calves are recorded lying for as much as 1020-1140 min/d (17-19 h/d) or as little as 364-735 min/d (6-12 h/d; Chua et al., 2002; Yanar et al., 2010; Stefanowska et al., 2002; Sutherland et al., 2013; Camiloti et al., 2012). It was evident from these values that calves exhibited a wide range of lying times, indicating that further study was needed to determine the relationship between calf comfort and lying time for dairy calves as well as the effect of management on comfort and lying time. Several factors in previous studies influenced calf lying times. Bedding material type influenced lying time as calves on organic surfaces spent as much as 776 minutes more lying down when compared to calves on inorganic surfaces (Stefanowska et al., 2002). Calves in the current study were all housed on inorganic materials, which may explain why they had lower lying times (917-980 min/d or 15-16 h/d) than calves housed on organic surfaces such as straw (lying times of 1020-1140 min/d or 17-19 h/d) in previous studies. Pre-weaned calves housed on a similar bedding material (river stones) spent around 1080 min/d (18 h) lying down, however, these calves were housed in groups, a factor which has previously been shown to increase lying time (Sutherland et al., 2013). A possible reason that calves spend more time lying on organic bedding surfaces is that these surfaces are often soft and absorbent. Housing type also influenced lying time with calves in group housing spent more time lying down than calves in individual housing, this effect was believed to be due to increased space allowance for calves in group housing (Tripon et al., 2012). Although previous research indicates that calves lie down more on a soft, dry surface, calves in the current study did not have increased lying times on sand or mats which provided a softer surface than gravel (Camiloti et al., 2012). However, previous research indicated a soft surface might not be necessary for lying behavior. Calves housed on river stones or sawdust did not differ in lying time at 1 wk of age or 6 wk of age (Sutherland et al., 2013). Our results were similar to those obtained when calves housed on either concrete or rubber mats did not differ in lying time with calves spending around 1013 min/d or 17 h/d lying down, which was close to the 917-980 min/d or 15-16 h/d of time spent lying observed in our calves (Hänninen et al., 2005). Calves on the current study may not have increased lying time on rubber mats or sand when compared to gravel because gravel provided good drainage and a dry surface. Also, because of mild temperatures throughout the study calves may not have required insulation from the ground provided by mats and somewhat by sand. In addition, calves in the current study were lighter weight than previously studied adult cattle and may not have required a soft surface for comfort (Drissler et al., 2005; Hänninen et al., 2005). Although lying time may not differ on different surfaces, a difference in lying behavior can still be observed. Bulls housed on concrete slats, rubber slats, and rubber mats had similar lying times, however, bulls on concrete slats had significantly more interruptions in lying behavior than those housed on rubber flooring (Graunke et al., 2011). One way to study lying behavior outside of lying time is by measuring lying bouts.

Lying bouts

Holstein calves may have had a greater number of lying bouts than Jersey calves because they were significantly larger and heavier and therefore may have felt more constrained by calf hutch hardware causing increased changes in position. Management was the same at both locations (feeding once per day) so it is unlikely that human interaction caused an increase in lying bouts in Holstein calves. Additionally, a similar effect was observed in adult dairy cattle housed on pasture or in stalls with cows in stalls showing a greater number of interruptions in lying behavior (Krohn and Munksgaard, 1993). Holstein calves had a slightly higher number of lying bouts/d than the 10-12 bouts/d observed in previous studies (Dellmeier et al., 1985). Jersey calves fell more closely in the range of 10-12 lying bouts/d regardless of treatment. It is possible that calves on the current study housed on mats had a higher number of lying bouts because the mats did not allow for drainage and became dirty; this effect was observed with calves housed on mats being much dirtier and wetter than those housed on gravel or sand. Interestingly calves had more variation in lying bouts during the first 3 wk of the study, this may have been due to calves adapting to their environment as the study progressed. Wet bedding was demonstrated to uncomfortable for calves and a dirty coat can cause discomfort and even wounds on the skin (Camiloti et al., 2012; Graunke et al., 2011). There is a limited amount of research which suggest that cattle may transition from lying to standing more in environments which were aversive (Krohn and Munksgaard, 1993). It is possible that this greater surface area caused Jersey calves to be more sensitive to heat loss by conduction through the wet flooring than Holstein calves.

Body weight, body weight gain, and grain intake

Holstein calves had a greater body weight and gained more weight than Jersey calves because of their larger frame size, a similar effect was observed in Holstein and Jersey calves born to pasture raised cows (Dhakal et al., 2013). These data indicated that calves gained weight and continued to grow throughout the study period regardless of bedding treatment. These data are consistent with previous research which determined that calves housed on river stones or sawdust did not differ in weight gain (Sutherland et al., 2013). This indicated that animals were healthy and were utilizing provided nutrients from milk and calf starter while undergoing rumen development (Berends et al., 2012b). Research suggested that Holstein calves' growth rate should not exceed 0.7 kg/d and Jersey calves' growth rate should not exceed 0.4 kg/d in order to reduce the risk of fat deposition in the mammary gland which can caused decreased milk production (Johnsson, 1988). Holstein calves met this recommendation as they gained appoximately 0.6 kg/d, Jersey calves also met the recommendation as they gained approximately 0.3 kg/d. It was important to note that this was a maximum recommendation and neither calf breed exceeded it, which indicated that they grew at an appropriate rate to reduce deposition of fat in the mammary.

The fact that calves continued to gain weight close to the recommended level even though the weather became increasingly colder suggested that calves were not negatively affected by environmental conditions and their intake remained sufficient to sustain growth (Appleman and Owen, 1971). Although our study was conducted during the fall and winter months, temperatures remained mild. High temperatures fell into the thermoneutral zone of calves (10-27° C with optimum comfort around 10° C), therefore it is unlikely that calves in the current study experienced enough cold stress to negatively affect growth on a bedding surface that did not allow for nesting (Godden, 2013).

Grain intake is closely related to weight gain and growth in calves because concentrate feeds such as calf starter allow for more dry matter intake and volatile fatty acid (VFA) production than roughages and presence of VFAs is essential for rumen development (Suárez et al., 2007). Holstein calves consumed more grain than Jersey calves because of their larger size and faster growth rate (Dhakal et al., 2013). Providing calves with access to solid feed is important to stimulate rumen development. Since calves in the current study consumed increasingly more grain and continued to gain weight as the study progressed they were able to successfully utilize nutrients from solid feed indicating satisfactory rumen development regardless of bedding treatment.

These data are similar to previous work where calf bedding surface did not influence grain intake or feed efficiency (Panivivat et al., 2004).

Cortisol and CBG

No literature is currently available on the differences between cortisol levels in dairy cattle breeds. One previous study noted that 2 breeds of beef cattle had significantly different basal cortisol concentrations (García-Belenguer et al., 1996). It is possible that Jersey calves naturally have higher plasma cortisol levels than Holsteins. Mean baseline cortisol levels in the current study are similar to previously recorded values for 4-6 wk old calves of a wide variety of breeds (Holstein, Jersey, Brown Swiss and crosses; Graf and Senn, 1999). No change in cortisol levels among treatments indicated that calves did not generate a physiological stress response to gravel, rubber mat, or sand bedding. No previous studies on the temporal pattern of cortisol in calves were found, however, data are available on age related changes in cortisol levels in other mammalian species. Cortisol levels in fetal sheep were increased near the time of parturition and this response was designed to induce labor in the dam (Wood and Keller-Wood, 1991). This may explain the high initial cortisol levels in calves on the current study. The linear pattern of decreasing plasma cortisol over time until around 6 wk of age was similar to that observed in piglets (Kattesh et al., 1990; Moya et al., 2007).

No data are currently available on age related changes in plasma CBG concentration in dairy calves, however, a study was conducted on age related change in plasma CBG in pigs (Roberts et al., 2003). Plasma CBG levels increased between 3 and 5 wk of age, which indicated that most plasma cortisol remained in the unbound form (available to the cell) during the first 3 wk of life (Roberts et al., 2003). Plasma CBG in calves began to increase most dramatically after 6 wk of age, which indicated, along with data presented for FCI, that calves had most plasma cortisol in the unbound form during the first 6 wk of life. This may indicate, as previously stated with pigs, that calves no longer need a source of readily available biologically active cortisol after 6 wk of age. This period before 6 wk of age may be a time of stress for calves as they are acclimating to a new environment and new stressors such as cold temperatures and disease.

One way that cortisol helps animal's cope with stress is by increasing blood glucose concentrations. Calves undergo the largest increase in rumen development around 4-6 wk of age, this is an additional reason that calves may need a higher level of cortisol before 6 wk of age as they adapt to absorbing nutrients from solid feed (Warner et al., 1956). A previous study in rats demonstrated that CBG levels decreased significantly following periods of stress initiated by hunger thereby increasing biologically active cortisol levels (Tinnikov, 1993). A longer period of study is needed to determine if plasma CBG levels remain constant in calves after 10 wk of age.

CONCULSIONS

There were no biologically significant differences in behavior or performance among bedding treatments. Calves had lying times and lying bout numbers consistent with literature recommendations regardless of bedding treatment, however, more research is needed to determine a consistent recommendation for calf lying times. It is likely that calves had adequate lying time because lying time is correlated with weight gain in pre-weaned calves and calves on this study gained weight at the recommended level as the study progressed regardless of treatment. Grain intake is associated with calf growth and calves on all treatments also consumed an increasing amount of grain as they approached weaning. There was no effect of bedding surface on cortisol levels indicating that calves did not show a physiological stress response to any of the bedding materials tested. Age related changes in cortisol are indicated by week effects and the pattern of age related change is similar to that previously shown in piglets. The reason for the pattern of cortisol levels may be due to the fact that calves required a higher available pool of cortisol prior to 6 wk of age. A possible reason for this is that calves undergo the largest change in rumen development from 4-6 wk and may need the additional cortisol for increased availability of glucose. These results as a whole suggest that bedding alone may not negatively affect welfare of pre-weaned Holstein and Jersey calves.

CHAPTER II
THE EFFECT OF MUSCID FLIES ON THE WELFARE OF PRE WEANED HOLSTEIN AND JERSEY CALVES

Abstract

Muscid flies were significant pests on dairy farms and negatively affected welfare of cattle and workers. Stable flies (*Stomoxys calcitrans L.*) had a painful bite and disrupted resting and feeding of dairy cattle, which had an economic as well as behavioral impact on dairy cattle production whereas house flies (*Musca domestica L.*) transmitted bacterial pathogens. Stable flies also decreased production in calves by reducing weight gain and house flies could spread diseases such as pinkeye to calves. Flies had previously been shown to reduce lying behavior in beef calves and a similar effect was predicted for dairy calves. The objective of this study was to determine the relationship between stable and house fly populations and the welfare of pre-weaned calves. Lying time, weight gain, and cortisol levels were assessed weekly in Holstein calves (from 9/18/2013-10/23/2013) housed at the Middle Tennessee Research and Education Center (MTREC) located in Spring Hill, TN and Jersey calves (from 9/18/2013-10/23/13) housed at the Dairy Research and Education Center (DREC) located in Lewisburg, TN. Calves were housed in plastic hutches and fed waste milk once/d; calf starter grain and water were offered *ad libitum*. For the final analysis 11 Holstein and 19 Jersey calves born between from September 2012 to October 2012 were utilized. Weekly response variables for overall calf welfare included lying behavior, lying bouts, weight gain, and plasma cortisol levels, and these were compared to muscid fly populations observed around the calf housing area. Lying times were evaluated using HOBO pendant-G dataloggers 6 d each week. Dataloggers were placed on the hind leg above the

pastern joint of calves. Loggers recorded at 1 min intervals and measured tilt which was converted to a position reading for calves (lying or standing). Loggers were removed and replaced each wk. Weight gain was determined by weighing calves at birth and each week. Blood was drawn at birth and once weekly using heparinized vacutainer tubes. Stress levels were measured using plasma cortisol analysis performed on weekly blood samples using a commercial RIA kit. Muscid fly populations were monitored using alsynite traps placed in the 4 cardinal directions of the calf area. During the period from 9/18/2013-10/23/13 an average of 5.4 ± 1.7 stable flies, 12.7 ± 5.5 house flies, and 17.5 ± 6.0 stable flies were collected at MTREC and 14.2 ± 6.0 stable flies, 17.2 ± 6.2 house flies, and 33.3 ± 6.3 total flies were collected at DREC. There was an inverse relationship between stable flies and lying time in Holstein calves and BW gain in Jersey calves. There was a tendency for an inverse relationship between house flies and BW gain in Holstein calves. There was an inverse relationship between total flies and BW gain in Holstein calves and total flies and lying time in Jersey calves. There was no relationship between fly populations and either breed of calf for plasma cortisol levels. These data indicate that populations of both stable and house flies negatively affected the welfare of pre-weaned Holstein and Jersey calves demonstrated by decreased lying time as well as decreased weight gain in dairy calves in response to increased stable and house fly populations. Since Muscid flies were a significant pest on dairy farms they negatively affected the welfare of dairy calves indicated by decreased lying times and weight gain in response to increased fly populations. These data indicate that increased populations of flies on dairy farms decreased the welfare and subsequent production of dairy calves.

Introduction

A major concern associated with intensive dairy production is that dairy farms are an ideal environment for the development of stable (*Stomoxys calcitrans L.*) and house flies (*Musca domestica L.*). Both types of fly develop in areas where manure is mixed with organic material such as straw or spilled feed (Christensen, 1982). These fly species cause animal welfare concerns indicated by animal irritation demonstrated by kicking and head tossing as well as decreased production (Christensen, 1982; Todd, 1964). Stable flies stress cattle because of their painful bites leading to decreased lying behavior and interrupted feeding which eventually decrease production (Christensen, 1982; Vitela et al., 2006; Taylor et al., 2012). Indicators of stress caused by flies in cattle include behavioral changes such as head tossing, foot stomping and bunching in attempts to dislodge or avoid flies, these behaviors lead to decreased lying time and decreased production (Todd, 1964; Vitela et al., 2006b). House flies are an issue on dairy farms because they can transmit disease organisms that cause pinkeye and mastitis, house flies also contaminated surfaces in the milking parlor with these pathogens (Christensen, 1982). Since both species of fly reproduced rapidly, fly populations on farm often increased very quickly (Christensen, 1982).

Since stable and house fly development on dairy farms is often in calf hutches and around 75 % of calves in the United States are housed in calf hutches it is important to address development of stable and house flies in this environment (USDA, 2007). A single calf hutch bedded with straw can produce 25,000-40,000 adult stable flies per summer (Schmidtmann, 1991). Stable and house flies that develop in calf hutches are not only a welfare concern for calves, they disperse to other areas of the farm and irritate adult cattle as well as farm workers (Schmidtmann, 1991).

Because stable and house flies can negatively affect animal welfare demonstrated by a decrease in natural behaviors such as lying behavior and production parameters such as weight gain it is important to evaluate Muscid fly populations with relation to parameters related to natural living, biological function (or production), and affective state of animals. Evaluation of lying behavior is used to assess comfort in dairy calves and adult cattle because it is considered an important indicator of comfort in dairy animals (Haley et al., 2000). Lying behavior may be altered by the irritating effects of flies. In one study, when there were more than 15 stable flies per animal, adult cows were very restless and unwilling to lie down due to constant attempts at dislodging flies (Todd, 1964). Although there is no data currently available on the effect of stable and house flies on lying behavior in dairy calves it is possible that stable flies also negatively affected lying behavior in dairy calves. Decreased lying behavior was not only an indicator of discomfort in cattle, it could also decrease production. For example, there was a tendency for decreased lying behavior to negatively affect weight gain in dairy heifers (Mogensen et al., 1997).

Beef cattle (approximately 14 months old) housed in pens kept fly-free gained 0.75 kg more than cattle housed in pens with stable flies (Campbell et al., 1977). In addition to cattle housed in confinement, stable flies also affected weight gain when cattle were housed on pasture. Yearling beef calves were maintained on pasture either with fly control or with exposure to stable flies. Calves exposed to stable flies experienced a 19% reduction in weight gain when compared to calves maintained with fly control (Campbell et al., 2001). It was likely that stable fly populations may have caused decreased weight gain and feed intake in these animals similar to the effects experienced in beef calves.

The affective state of animals was described as how the animal “feels” and included states such as fear, pain, or stress (Fraser, 2008). Stable flies negatively altered the affective state of cattle by causing discomfort and restlessness (Christensen, 1982); Rutz et al., 2010). Cattle expressed physiological signs of stress such as elevated heart rate and respiration rate when exposed to ten or more stable flies per animal for 1 h (Schwinghammer et al., 1987). When cattle were exposed to around 25 stable flies/animal for 1 h an increase in the stress hormone cortisol was observed (Schwinghammer et al., 1987).

Although there were many studies quantifying the effect of fly populations on the welfare of adult dairy cattle and beef calves, there was little information available about the effect of fly pressure on the welfare of pre-weaned dairy calves. In addition most studies on fly pressure were performed during peak fly populations (Spring and Summer months), however, it remains to be known how Muscid flies affect farms which practice Winter calving, a common practice in the Southeast. This study focused on the effects of fly populations on the overall welfare of pre-weaned dairy calves born during Fall and Winter months (calves born from September to March). The objective of this study was to determine the relationship of stable and house fly populations to the behavior, performance, and affective state of Holstein and Jersey calves housed in individual hutches. This is important because it allows us to examine the effects of Muscid flies on calves born when fly populations were not at peak. Information on these effects will help define future practices on farms regarding fly control during months when flies are not at peak.

Materials and methods

Animals and management

A total of 23 Holstein and 38 Jersey calves born between September 2012 and March 2013 were enrolled in this study (last sampling date 4/9/2013). For the final analysis, a subset of 11 Holstein and 19 Jersey calves born between from September 2012 and October 2012 were utilized. This subset of calves was chosen because fly populations were highest during this period. All calves were housed in commercial plastic calf hutches (Calf-Tel, Hampel Animal Care, Germantown, WI) with an outside wire enclosure and racks to hold buckets for starter grain and water. Calf starter utilized at both farms contained 18 % protein

2 % crude fat (tag values, Tennessee Farmers Co-op, LaVergne, TN). All calves were fed 4 L waste milk once daily and water was provided *ad libitum*. Calves were assigned to one of three hutch bedding treatments: gravel (typically 2.5 cm in diameter), rubber mat (1.3 cm thick) over gravel base, or river sand (5 cm deep) over a gravel base. All animal procedures were approved by the University of Tennessee IACUC committee (protocol #2117-0712).

Holstein calves were housed at the Middle Tennessee Research and Education center (MTREC) in Spring Hill, TN. Calf hutches at MTREC were placed on a gravel pad (gravel was approximately 2.5 cm in diameter) measuring approximately 12 m × 54 m**.** Calf hutches measured 2.1 m × 1.2 m inside and had an attached outside area measuring 1.1 m × 1.7 m surrounded by a 1.3 m high wire fence. Holstein calves were offered 0.21 ± 0.02 kg calf starter initially which was increased by 0.2 kg increments as needed to ensure *ad libitum* access. Holstein calves were weaned at 6.5 ± 0.2 wk of age.

Jersey calves were housed at the Dairy Research and Education Center (DREC) in Lewisburg, TN. The DREC calf hutches were placed on a gravel pad measuring approximately 21 m × 61 m. Calf hutches measured 2 m × 1.2 m inside and had an attached outside area measuring 1.5 m × 1.2 m surrounded by a 1.2 m high wire fence. Jersey calves were fed 4 L waste milk until 2 wk prior to weaning when milk was reduced to 2 L once/d. All Jersey calves were offered 0.2 kg calf starter initially which was increased by 0.2 kg increments as needed to ensure ad libitum access. All Jersey calves were weaned at 10 wk of age.

Evaluation of fly population

Several methods were attempted to measure fly populations. Bedding collections were collected from gravel and sand hutches, however, only 1 fly larvae was found so this approach was not continued. Leg counts to determine number of stable flies on calves were performed, but discontinued due to the presence of very few flies. Spot cards were placed in hutches to determine house fly activity, but were dicontinued after frequent disruption by weather and calves. Muscid fly populations were successfully monitored using four Olson type alsynite traps (Olson Products, Medina, OH) placed at four ordinal locations around the calf hutch area. Traps were located approximately 1-2 m from the closest calf hutch. This type of trap has been optimized for trapping stable flies but will also collect house flies (Taylor and Berkebile, 2006). Traps were covered with sticky sheets and stable and house flies counted weekly. Sticky sheets were replaced each time flies were counted. Although most previous studies determine fly populations on an individual animal basis, our method of using traps assessed overall fly pressure and was suitable for detection of effects of weekly fly pressure on overall animal welfare (Campbell et al., 2001;Taylor et al., 2012).

Lying behavior

Mean lying time (min/d) was determined using the HOBO pendant G accelerometer data logger (Onset Corporation, Bourne, MA; Bonk et al., 2013). Data loggers were attached to the lateral side of calves' hind legs above the pastern joint with vet wrap. Calves were fitted with a logger after birth during the move to individual hutches. Loggers were removed and replaced each week on alternating legs to prevent skin irritation. Loggers recorded posture at 1-min intervals for 6 d/wk throughout the study. Lying time was calculated as described by (Bonk et al., 2013).

Biological function

On the day of birth, the time of birth, birth weight, and time of first colostrum were recorded for all calves. Calves were weighed weekly until weaning to determine body weight gain using a mobile scale (Tru-Test, Tru-Test Limited, Auckland, NZ). Calf health was evaluated by farm staff at each location according to the University of Wisconsin calf health scoring chart guidelines (Lago et al., 2006). Type and duration of any treatment administered to calves was recorded.

Plasma cortisol levels

Blood samples were collected from all calves on the day of birth and weekly until weaning. Approximately 10 mL of blood was collected via jugular venipuncture into a heparinized vacutainer tube (BD vacutainer, Franklin Lakes, NJ). Blood samples from Jersey calves were taken with calves in hutches, while Holstein calves were moved approximately 1.5 m from the housing area to a mobile head catch for blood sampling. Care was taken to obtain a sample in 2 min or less to decrease calf stress and the possibility of elevated plasma cortisol concentrations. Vacutainer tubes were spun at 3,000 × *g* for 10 min immediately after sampling was completed. Separated plasma was aliquoted into two 2 mL cryogenic vials with a disposable transfer pipette and stored at -20 °C until analysis. Plasma total cortisol concentrations were determined on duplicate samples using an RIA procedure (Coat-A-Count, Diagnostic Products, Los Angeles, CA). Samples were analyzed in duplicate and counted for 1 min using a gamma counter (Wizard Automatic Gamma Counter, PerkinElmer, Waltham, MA). Plasma cortisol concentrations < 3 ng/mL were considered to be below detectable limits. Intra- and inter-assay CV was determined for low (10 ng/mL), medium (30 ng/mL), and high (50 ng/mL) bovine cortisol standards. Intra-assay CVs were 10.7%, 5.6%, and 6.6% for low, medium, and high standards respectively. Inter-assay CVs were 12.3%, 9.6%, and 7.1% for low, medium, and high standards respectively.

Statistical analysis

The effect of fly population (stable, house, or all flies) on mean lying time, body weight gain, and cortisol levels for the first 6 wk of the study and the entire study using linear regression in SAS (SAS Institute, Cary, NC) data are reported for the first 6 wk. Calves less than 2 wk of age were excluded from the plasma cortisol analysis because of high cortisol levels during the first 2 wk of life, this reduced the chance of age relative to birth being a confounding variable for plasma cortisol levels (Adcock et al., 2007). Cortisol data were log transformed. The effect of calf age on lying time, body weight gain, and cortisol levels was also determined using linear regression. Differences were declared significant at $P < 0.05$ and trends were declared at $P < 0.09$.

Results

Fly populations

At MTREC (Holsteins), stable fly populations were relatively low compared to house flies and were highly variable with fluctuations from a low of 1.8 ± 1.6 flies during the week of October 2nd to a high of 8.3 ± 1.9 flies during the week of October 16th. House fly population peaked at 26.8 ± 5.1 flies during the week of October 2nd and remained relatively consistent before decreasing to 4.0 ± 5.1 flies during the week of October 23rd.

At DREC (Jerseys), stable fly populations were greater than at MTREC, but followed a similar highly variable pattern. Stable fly population peaked at 34.3 ± 5.9 flies during the week of September 25th. Population then decreased to 7.0 ± 6.8 flies during the week of October 2nd before increasing during the 2 subsequent weeks and ultimately decreasing to 6.75 ± 5.9 flies during the week of October 23rd. House fly population s peaked at 39.5 ± 6.0 flies during the week of October 9th before decreasing to 10.0 ± 6.0 flies during the week of October 16th and increasing slightly to 12.3 ± 6.0 flies during the week of October 23rd.

Lying time

There were no relationships between total fly populations, stable fly populations, and lying bouts in Holstein or Jersey calves (data not presented). There was no relationship of total fly populations to lying behavior for Holstein calves (P = 0.75, R^2 = 0.04; data not presented). However, there was an inverse relationship between stable fly populations and lying time. When stable fly populations were high, lying times were low (P = 0.03, R^2 = 0.82; figure 9; all figures are located in the appendix). There was no relationship of house fly population to lying time (*P* = 0.93; R^2 = 0.003; data not presented). Jersey calves had a mean lying time of 926.5 ± 25.3 min/d and there was an inverse relationship between total flies and lying time. When total fly populations were high lying time was lowest (*P* = 0.03, R^2 = 0.83; figure 10), but no effect of stable flies alone (*P* = 0.14, R^2 = 0.58; data not presented) or house flies alone (*P* = 0.59, R^2 = 0.11; data not presented). Age of calves was not related to lying time in either Holstein (*P* = 0.77) or Jersey (*P* = 0.95) calves.

Body weight gain

Holstein calves had a mean body weight gain of 4.0 ± 1.2 kg/wk and there was a relationship of total fly populations to BW gain (*P* = 0.01, R^2 = 0.81; figure 11). There was no relationship of stable flies (*P* = 0.20, R^2 = 0.37; data not presented). There was a trend for an inverse relationship of house fly populations to BW gain. As house fly populations increased BW gain decreased this relationship may have been driven by the fact that house fly populations were greater than stable fly populations at MTREC (*P* = 0.05; R^2 = 0.66; figure 12). Jersey calves had a mean body weight gain of 3.2 ± 1.1 kg/wk. There was no relationship of total fly population to body weight gain in Jersey calves (*P* = 0.30; R^2 = 0.30; data not presented). An inverse relationship was present between stable fly population and body weight gain. When stable fly populations were high, BW gain was low (*P* = 0.03; R^2 = 0.75; figure 13) in Jersey calves. There no relationship between house fly populations and body weight gain in Jersey calves

($P = 0.66$; $R^2 = 0.05$; data not presented). Age of calves was not related to body weight gain in Holstein calves ($P = 0.13$; $R^2 = 0.07$). There was a significant relationship (as age increased BW gain increased) between age of calves and body weight gain in Jersey calves but it was weak ($P = 0.004$; $R^2 = 0.17$).

Plasma cortisol levels

Holstein calves had a mean plasma cortisol level of 17.8 ± 3.5 nmol/L (untransformed). There was no relationship of total flies ($P = 0.50$; $R^2 = 0.04$; data not presented), stable flies ($P = 0.64$; $R^2 = 0.02$; data not presented), or house flies ($P = 0.58$; $R^2 = 0.02$; data not presented) to plasma cortisol levels in Holstein calves. Jersey calves had a mean plasma cortisol level of 33.1 ± 5.8 ng/mL (untransformed). There was no relationship of total flies ($P = 0.57$; $R^2 = 0.04$; data not presented), stable flies ($P = 0.14$; $R^2 = 0.04$; data not presented), or house flies ($P = 0.06$; $R^2 = 0.04$; data not presented) to plasma cortisol levels in Jersey calves. Age was not related to plasma cortisol levels for Holstein calves ($P = 0.40$; $R^2 = 0.05$). For Jersey calves, there was a significant relationship between age and plasma cortisol levels, however the R^2 showed a weak relationship ($P < 0.01$; $R^2 = 0.16$).

Discussion

This study was unique in that it was performed during months of the year mostly outside of periods where stable and house fly populations are highest in Tennessee. Calves were housed on inorganic bedding surfaces which are associated with significantly less fly production than organic surfaces such as straw which explains why no fly larvae were successfully found in bedding (Schwinghammer et al., 1987). Because of lower fly populations, the traditional method of evaluating fly pressure based on counting flies on individual animals was unsuccessful (Taylor et al., 2012). Using spot cards to track house fly activity inside hutches also did not yield any results because calves and weather events disturbed the cards. Alsynite traps were chosen to evaluate fly populations around the calf area because they consistently trapped flies even during the coldest weeks of the study. The traps were placed out of reach of calves and were rarely disturbed by weather events, such as high winds.

Fly populations

Fly populations at both farms were similar to expected populations for Fall and Winter, according to the Tennessee Dairy Cattle Pest Control Profile (Stewart, 2007). Stable fly populations are heaviest from March-May, however, house fly populations are highest from early March-October (Stewart, 2007). Observations on fly populations in the current study were similar to patterns observed by Stewart in Tennessee. House fly populations were much higher than stable fly populations at MTREC. This was expected because house fly populations in Tennessee remain high into the month of October typically. Interestingly, stable fly populations remained higher than house fly populations at DREC even though peak populations for stable flies usually occur during the spring and early summer. This is possibly because the DREC facility had more potential stable fly breeding sites, such as spilled feed and manure left in sheltered areas such as under gates than the MTREC facility.

Economic impacts such as decreased weight gain and milk production may occur when there are greater than 10 stable flies per cow (Taylor et al., 2012). It was not clear in our study how many flies were affecting each individual animal because performing leg counts, a procedure normally used to determine the number of flies per calf, did not yield any flies. As mentioned previously, detection of house flies affecting individual animals by the use of spot cards and detection of fly larvae in bedding material for individual animals were also unsuccessful. In the future, it would be beneficial to develop a successful method for evaluating fly pressure on individual animals during periods when fly populations are low because it appears that even fewer flies than previously thought adversely influenced the behavior and performance of calves. It would also be of merit to repeat the current study during a period of peak fly populations because some farms, such as those that rely on grazing may not be able to avoid having calves during peak fly populations.

Lying time

For Holstein calves, mean lying time was inversely related to mean stable fly populations with lowest weekly mean lying times occurring at points where weekly mean stable fly populations were greatest. For Jersey calves, there was an inverse relationship between stable and house fly populations and mean lying time, with minimum lying times occurring at the same points as maximum fly populations. These data indicated that high fly populations negatively impacted that welfare of Holstein and Jersey calves by decreasing lying behavior, which is considered essential for animal comfort of dairy cows and calves (Haley et al., 2000; Chua et al., 2002).

There is little data available on the relationship of flies to lying behavior in dairy calves. However, stable flies reduced lying behavior in adult cattle in a similar manner to calves observed in the current study. Dairy cattle housed outdoors in New Zealand showed extreme irritation and no attempts to lie down when there were 15 stable flies/cow or more, which indicated stable flies negatively influenced lying behavior (Todd, 1964). In a study of 80 Holstein cows receiving either no treatment or an insecticide treatment and given access to 4 pen areas (feeding area, drinking area, covered area, manure area), insecticide treatment did not affect number of cows lying down (Vitela et al., 2006b). However, cows without insecticide treatment altered their lying behavior by spending more time lying in manure in an attempt to protect legs and underbellies from stable fly bites (Vitela et al., 2006b). These data indicate that controlling fly population may lead to better animal cleanliness and animal comfort in adult cattle, a similar effect is likely in dairy calves as well (Vitela et al., 2006b).

Body weight gain

Mean body weight gain in Holstein calves was inversely related to total fly populations with minimum weight gain occurring at maximum stable and house fly population and vice versa, the same effect was evident for house flies. This effect may have been driven by the fact that house fly populations were higher than stable fly populations at MTREC.

There was an inverse relationship between stable fly populations and body weight gain in Jersey calves with minimum weight gain occurring during maximum stable fly populations. This relationship may have been driven by higher stable fly than house fly populations at DREC.

It is possible that a relationship between stable flies and body weight gain was observed in Jersey calves, but not Holstein calves, because stable fly populations overall were higher at DREC than at MTREC. This is in accordance with a previous study that determined there was a larger effect on weight gain when cattle were exposed to 100 flies vs 50 flies per animal (Campbell et al., 1977). Although these animals were exposed to many more flies than calves on our study, it indicated that as fly populations increase, negative effects associated with stable flies increase (Campbell et al., 1977). Although our data did not show a consistent effect of fly species across the two calf breeds, the relationship between stable fly populations and body weight gain in Jersey calves was similar to data from the literature indicating that stable fly pressure causes decreased weight gain in calves (Campbell et al., 1977; Campbell et al., 2001). Available data indicate similar effects, with high fly populations causing decreased weight gain in calves. For example, 14 month old heifers housed in a feedlot setting had a decrease in BW gain when exposed to a stable fly population in a screened in pen maintained at 50 flies/calf (Campbell et al., 1977). In steers housed on pasture, a group of steers maintained at 5 stable flies per front leg using scheduled fly releases had reduced weight gain when compared to a group of steers maintained with fly control. Interestingly when both groups were moved from pasture to a feedlot steers exposed to flies did have compensatory gain during an 84 d feeding period (Campbell et al., 2001). This suggested exposure to fly pressure early on can negatively affect calves even after flies are removed (Campbell et al., 2001).

Plasma cortisol levels

Plasma cortisol levels in this study were similar to values previously recorded for 4-6 week old calves (Holstein, Jersey, Brown Swiss and crosses) not subjected to pain or stress (approximately 10-30 nmol/L; Graf and Senn, 1999). When subjected to stress from a painful procedure (dehorning), cortisol levels in these animals increased to approximately 50-80 nmol/L (Graf and Senn, 1999). Although fly pressure elicited increases in cortisol concentrations of cattle, it was likely that calves in the current study were not exposed to sufficient fly populations. In a previous study, stable flies were allowed to feed on steers through the use of cages attached to the animal. Even at a population of 30 stable flies/animal, no difference in cortisol concentration was observed (Estienne et al., 1991). Serum cortisol levels were increased by exposing 1 year old steers to 100 horn flies plus 25 stable flies per animal as well as 500 horn flies plus 50 stable flies per animal using cages strapped to animals to ensure fly feeding (Schwinghammer et al., 1987). Although we could not directly evaluate the number of flies/animal in our study, it was likely that they did not exceed 25-50 flies/animal based on fly population data obtained weekly. Therefore it was unlikely that cattle in our study were exposed to sufficient number of flies to increase cortisol levels. However, it is important to note that even though cortisol levels may not increase in response to flies a production or behavioral response was observed at 5-15 stable flies/animal (Campbell and Berry, 1989; Todd, 1964).

Conclusions

Stable and house flies negatively influenced the welfare of both Holstein and Jersey calves. High populations of flies decreased mean lying time, an indicator of discomfort and annoyance as well as body weight gain. Fly populations did not affect plasma cortisol levels in dairy calves. More consistent effects on lying behavior and body weight gain may have been observed if the study was conducted during peak fly season in Tennessee (from March to May) when higher fly populations could be recorded. Most previous experiments on the effect of flies on animal welfare were performed during periods of peak fly populations. One unique aspect of our study is that it was performed during months when fly populations were not necessarily at their peak. Our data are valuable in that they indicate that flies still negatively influence calf behavior and growth even when fly populations are not at a peak. Although using traps was successful because significant relationships between fly populations and measures of animal welfare were present the importance of measuring stable and house fly pressure on individual animals should not be overlooked.

CONCLUSION

The first study demonstrated that gravel, rubber mats, and sand could all be used as bedding materials for pre-weaned calves without causing a decrease in calf welfare. There was a significant effect of breed for all response variables so data from Holstein and Jersey calves were analyzed separately. There was no effect of bedding treatment on lying time, lying bouts, body weight, weight gain, grain intake or cortisol levels in Holstein calves. There was no effect of bedding treatment on any of these response variables in Jersey calves with the exception of Jersey calves housed on rubber mats having a significantly higher number of lying bouts than Jersey calves housed on gravel or sand. Lying bouts were also much more variable during the first 3 wk of life, possibly indicating that calves were adapting to their environment. An additional component to this study involved determining the levels of corticosteroid binding globulin (CBG) in Jersey calves. There was no effect of treatment on CBG levels, a time effect was evident with a decrease in CBG around 6 wk of age. However, this time period corresponded with a decrease in milk allowance and may have been in response to this diet change. A similar effect was observed in rats with decreased food allowance. Changes in CBG over time were evaluated in pigs and had high initial levels similar to the calves before decreasing to a baseline level and undergoing an increase around 4 wk of age possibly due to the start of production of CBG in the liver. A similar effect may occur in calves subjected to a longer sampling interval than was utilized in the current study.

More consistent differences in behavior and performance among bedding treatments would have been observed if temperatures were consistently lower (< 10° C) during the study period. Although this study was performed during the fall and winter months, temperatures remained mild with high temperatures remaining within the thermoneutral zone of calves (10-27° C) for most of the study period. In the future, it would be interesting to measure skin surface temperature of calves as well as bedding surface temperature to determine which surfaces caused the most heat loss by conduction. Another possible future study would be to include an organic bedding material treatment, such as straw, to determine if this type of bedding could increase calf welfare relative to inorganic bedding. Overall, this study showed that gravel, rubber mats, and sand were all suitable bedding materials for pre-weaned calves indicated by the fact that there were no biologically significant effects of bedding on natural living, biological function, or affective state of calves.

The second study demonstrated that as stable and house fly populations increased welfare of pre-weaned Holstein and Jersey calves decreased. Stable and house fly populations were inversely related to lying time in Jersey calves but not Holstein calves. Stable fly populations were inversely related to lying time in Holstein calves but this relationship was not evident for Jersey calves. Total fly populations were inversely related to body weight gain in Holstein calves but not Jersey calves. Stable fly populations were inversely related to body weight gain in Jersey calves but not Holstein calves. Interestingly, house fly populations were inversely related to body weight gain in Holstein calves with no relationship in Jersey calves. Fly populations did not affect cortisol levels in either calf breed. Graphs of data for these relationships showed similar numeric patterns with decreased lying time and body weight gain occurring during periods of increased fly populations even when relationships were not statistically significant. As mentioned previously, fly populations were likely not sufficient to cause an increase in cortisol levels.

In the future, it would be interesting to repeat this study during a period of peak fly populations, this approach would allow for individual sampling of fly pressure on calves. It is important to study the effects of peak fly populations on calves because not all farms are able to restrict calving to months when fly pressure is lessened. Examples include larger farms with year round calving as well as grazing dairies which rely on cows calving during peak grazing season. Using organic bedding would also be beneficial as inorganic beddings such as the materials we used have been associated with significant decreases in fly development. Organic beddings warrant study because they are sometimes required to insulate calves in cold temperatures. Finally, a house fly specific trap could be implemented as alsynite traps are designed to attract stable flies. A house fly specific trap would allow for more accurate counting of house flies and a better assessment of overall house fly populations.

Both of these studies demonstrated the need to further investigate the effects of dairy calf management on animal welfare. Our study demonstrated that on a well-managed farm gravel, rubber mats, and sand could be used as bedding for pre-weaned calves without negatively affecting animal welfare. The second study demonstrated that stable and house flies decreased calf welfare even when fly populations were not at peak. We did not compare these inorganic surface to an organic surface such as straw which may have provided a softer surface for calves, however, it would also likely increase fly pressure. Future work may include comparing welfare of calves housed on organic materials such as straw with our data on calves housed on inorganic materials. Future work on the effect of fly pressure may include comparing data obtained during peak fly season with our data along with utilizing different methods to evaluate fly populations.

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Appendix

Table 1. Bedding material costs

Bedding Type	Cost*	Cost per hutch
Gravel	$244.00	$10.17
Rubber Mat	$1114.24	$46.42
Sand	$546.62	$22.78

*Includes hauling

Table 2. Summary of effects of flies

Response Variable	N=	Mean No. Stable flies	Statistic	Mean No. House flies	Statistic	Mean No. Total flies	Statistic
Holstein calves							
Lying time	11	5.4 ± 1.7	*P* = 0.03; R^2 = 0.82	12.7 ± 5.5	NS	17.5 ± 6.0	NS
Lying bouts	11	5.4 ± 1.7	NS	12.7 ± 5.5	NS	17.5 ± 6.0	NS
Weight gain	11	5.4 ± 1.7	NS	12.7 ± 5.5	*P* = 0.05; R^2 = 0.66	17.5 ± 6.0	*P* = 0.01; R^2 = 0.81
Cortisol	11	5.4 ± 1.7	NS	12.7 ± 5.5	NS	17.5 ± 6.0	NS
Jersey Calves							
Lying time	19	14.2 ± 6.0	NS	17.2 ± 6.2	NS	33.3 ± 6.3	*P* = 0.03; R^2 = 0.83
Lying bouts	19	14.2 ± 6.0	NS	17.2 ± 6.2	NS	33.3 ± 6.3	NS
Weight gain	19	14.2 ± 6.0	*P* = 0.03; R^2 = 0.75	17.2 ± 6.2	NS	33.3 ± 6.3	NS
Cortisol	19	14.2 ± 6.0	NS	17.2 ± 6.2	NS	33.3 ± 6.3	NS

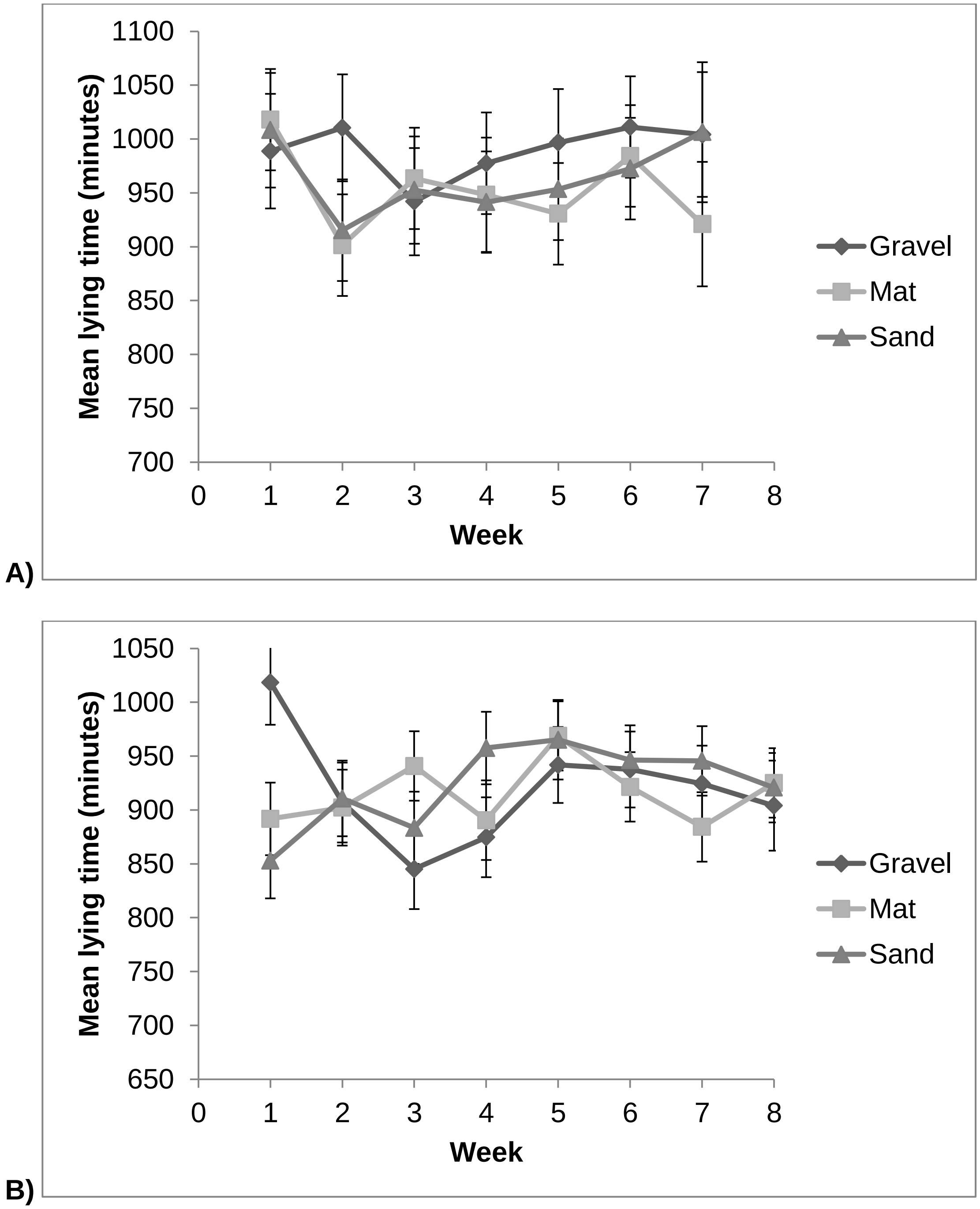
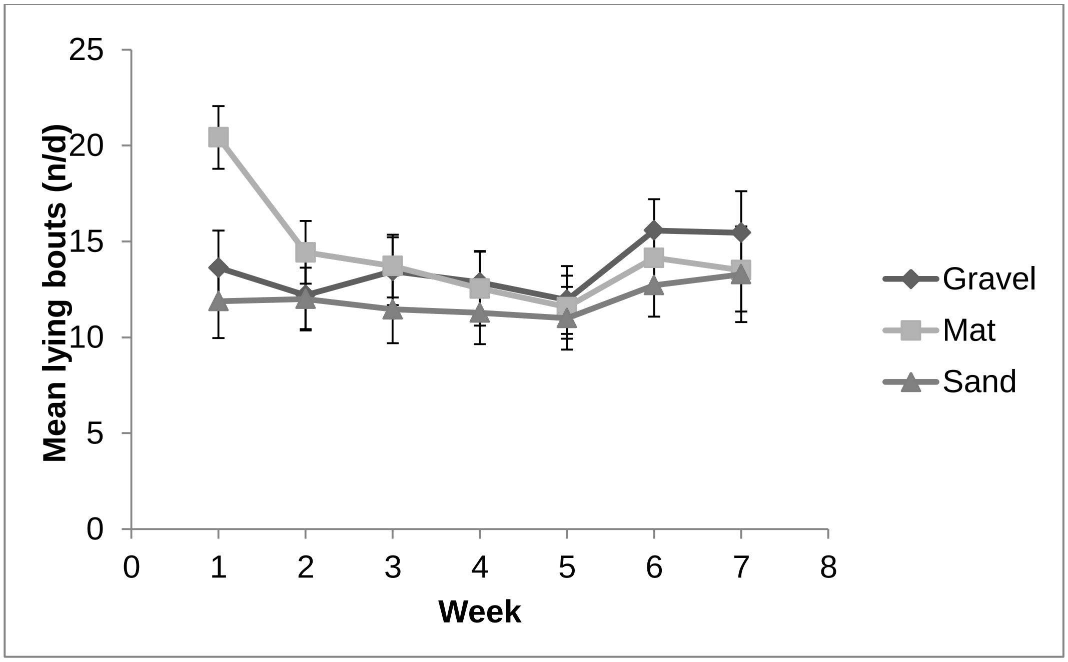
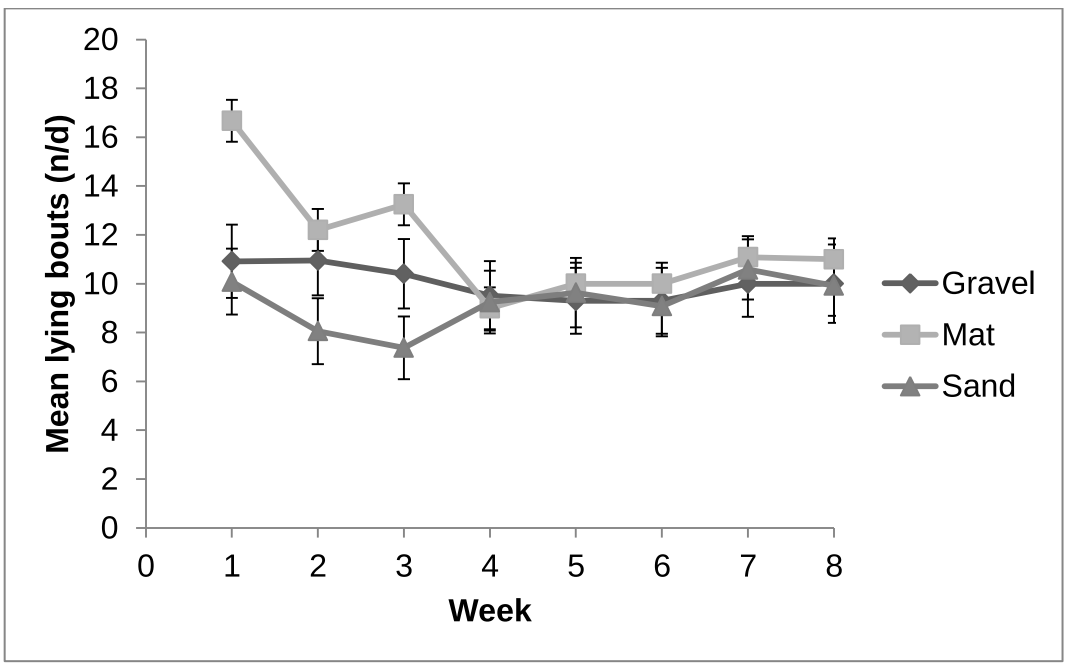


Figure 1. Mean lying time of Holstein calves (A). Mean lying time of Jersey calves (B).

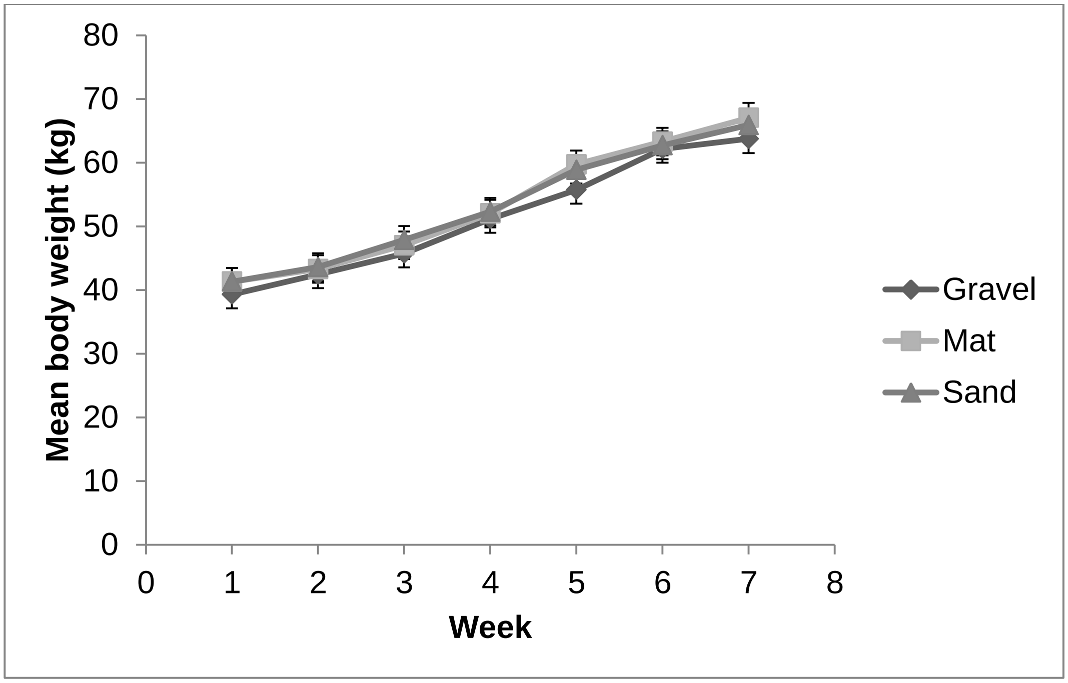


A)

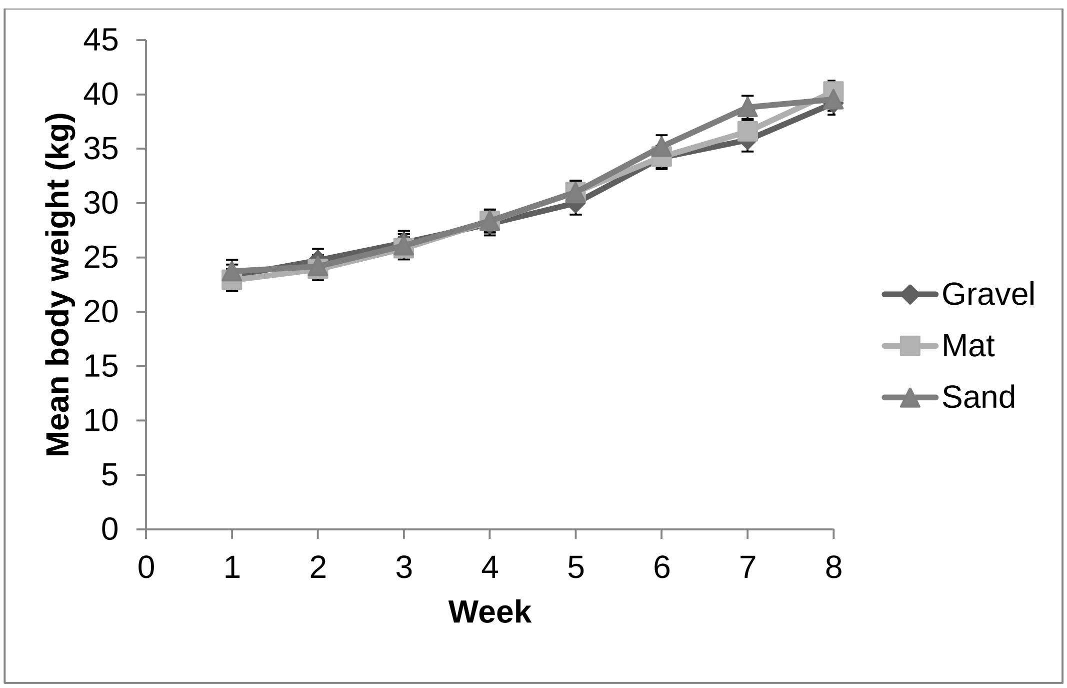


B)

Figure 2. Mean lying bouts of Holstein calves (A). Mean lying bouts of Jersey calves (B).

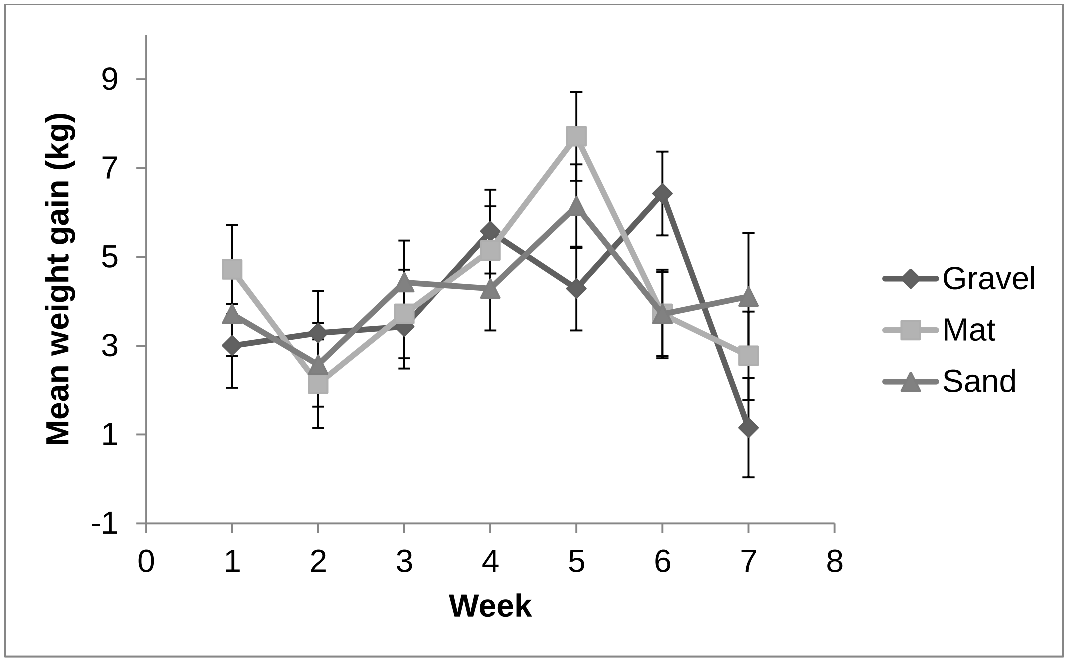


A)

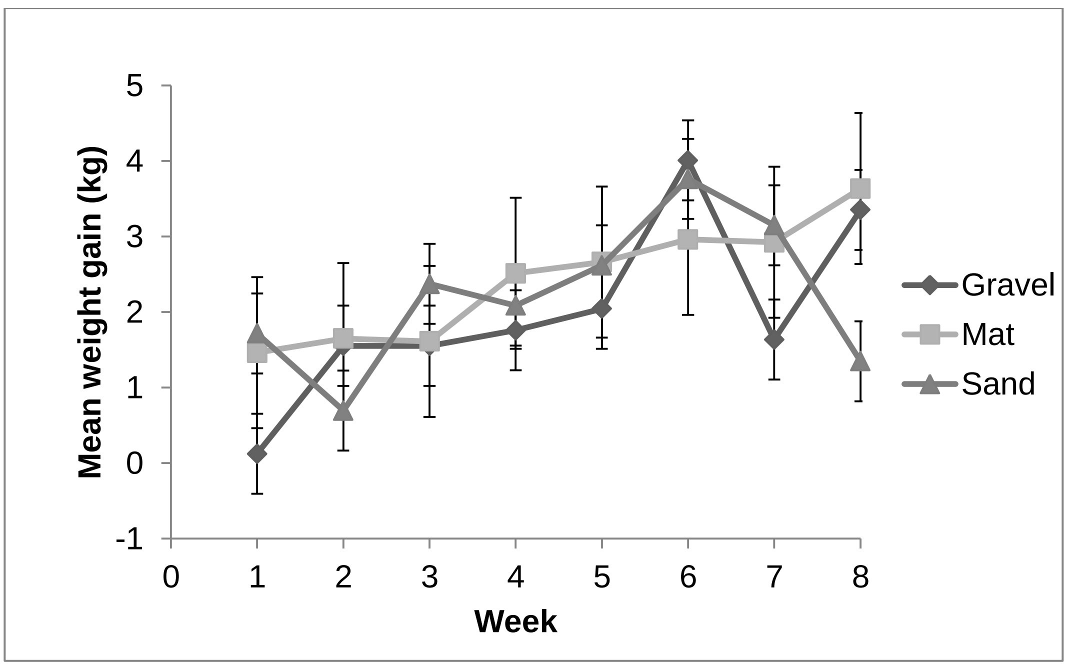


B)

Figure 3. Mean body weight of Holstein calves (A). Mean body weight of Jersey calves (B).

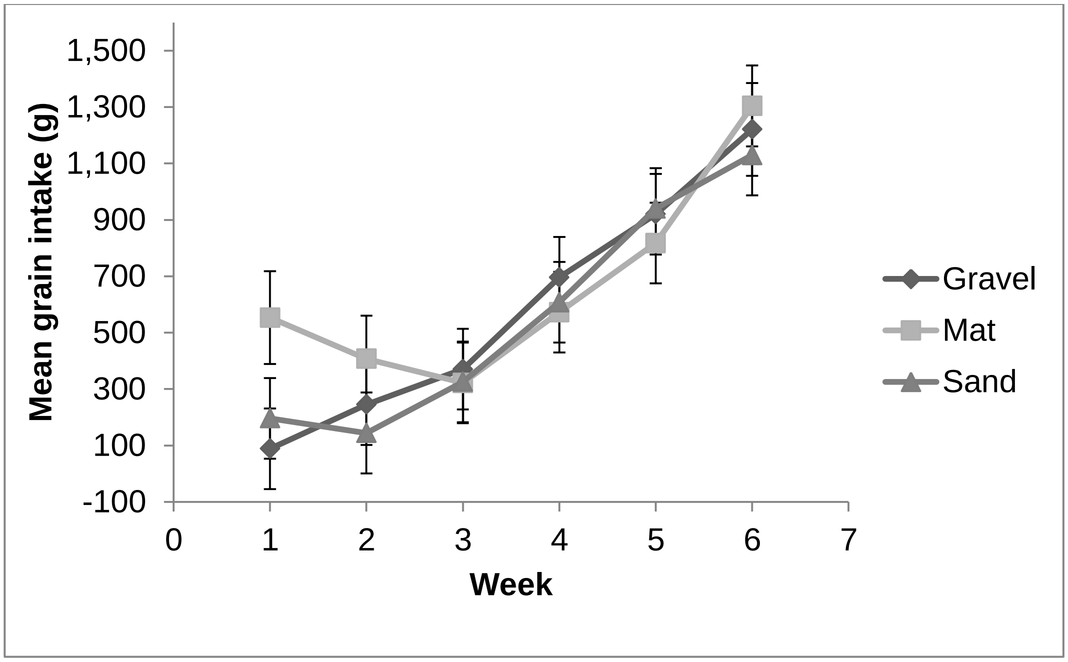


A)

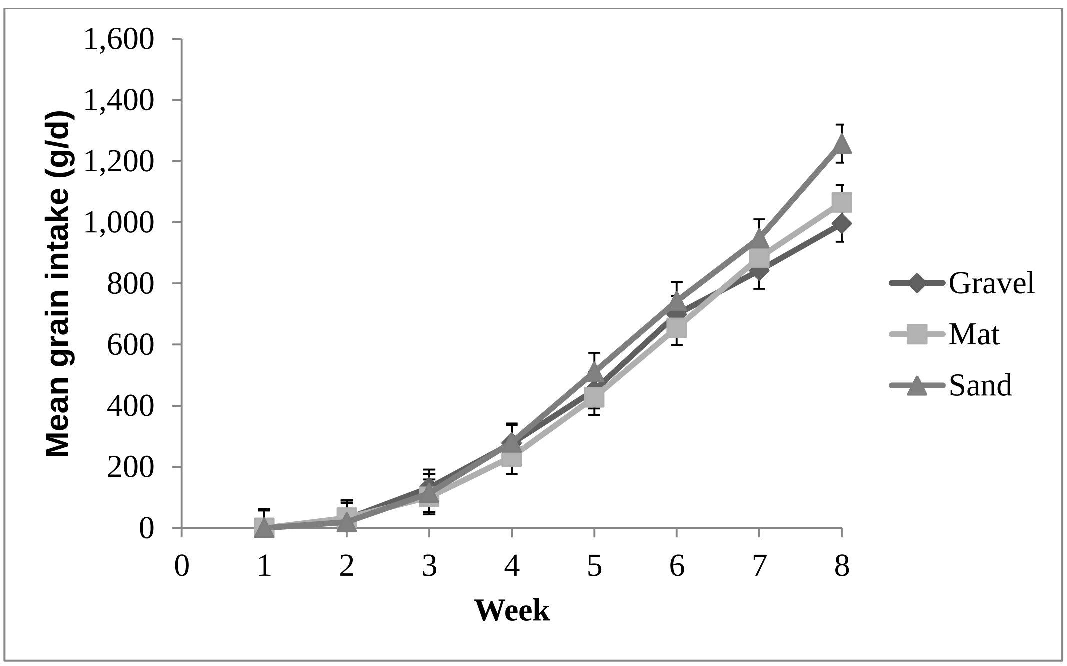


B)

Figure 4. Mean body weight gain of Holstein calves (A). Mean body weight gain of Jersey calves (B).

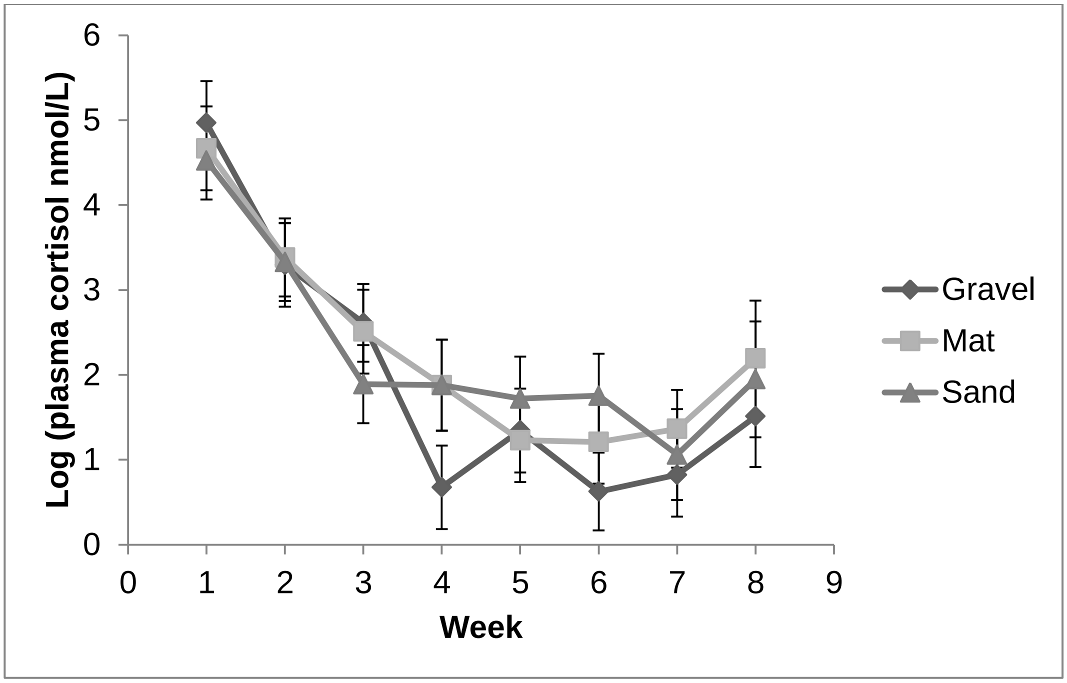


A)

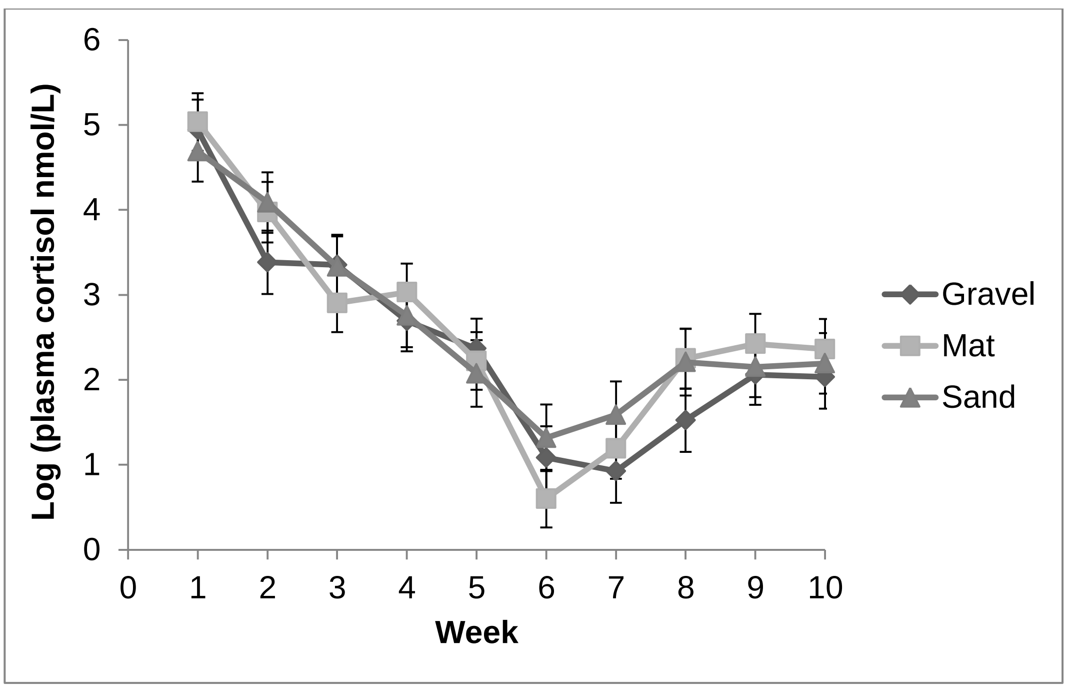


B)

Figure 5. Mean grain intake of Holstein calves (A). Mean grain intake of Jersey calves (B).

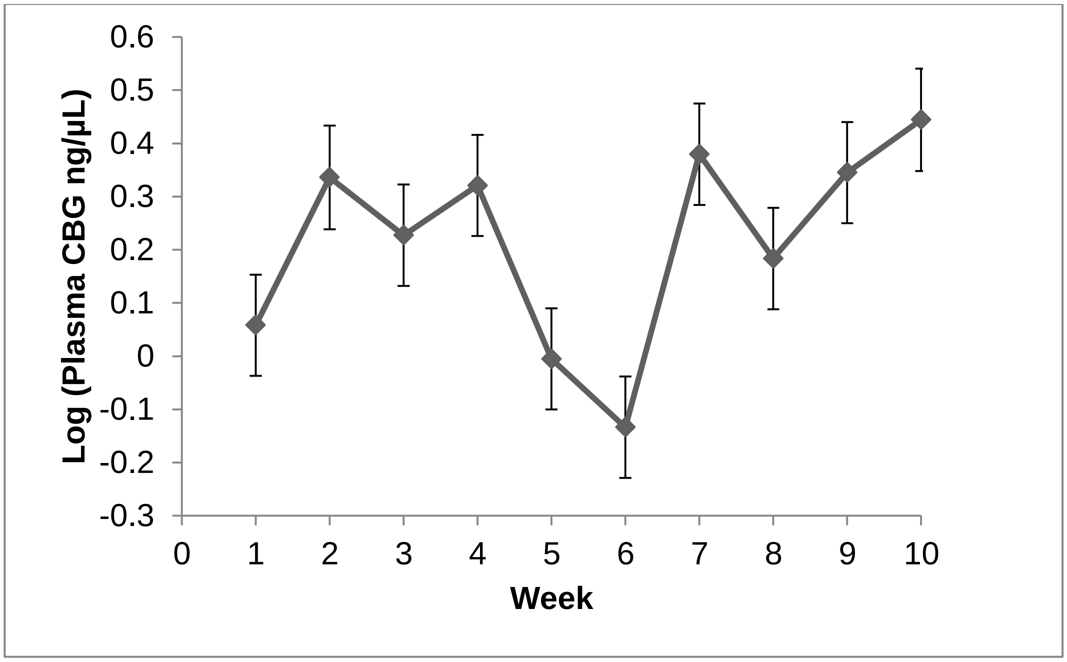


A)

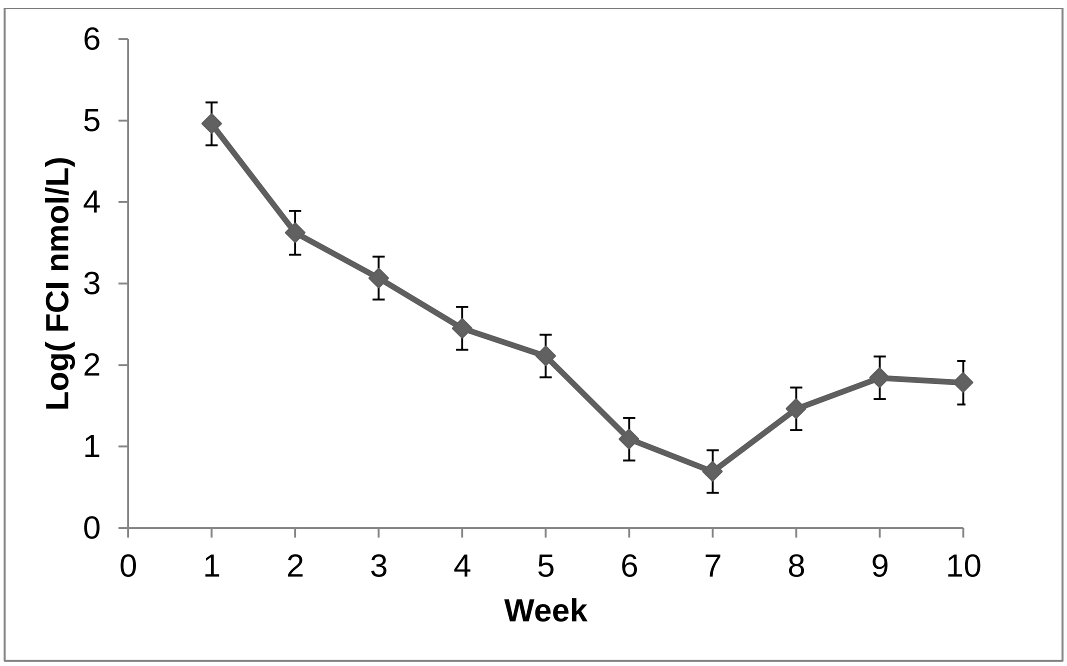


B)

Figure 6. Mean cortisol levels of Holstein calves (log transformed) (A) Mean cortisol levels of Jersey calves (log transformed) (B).

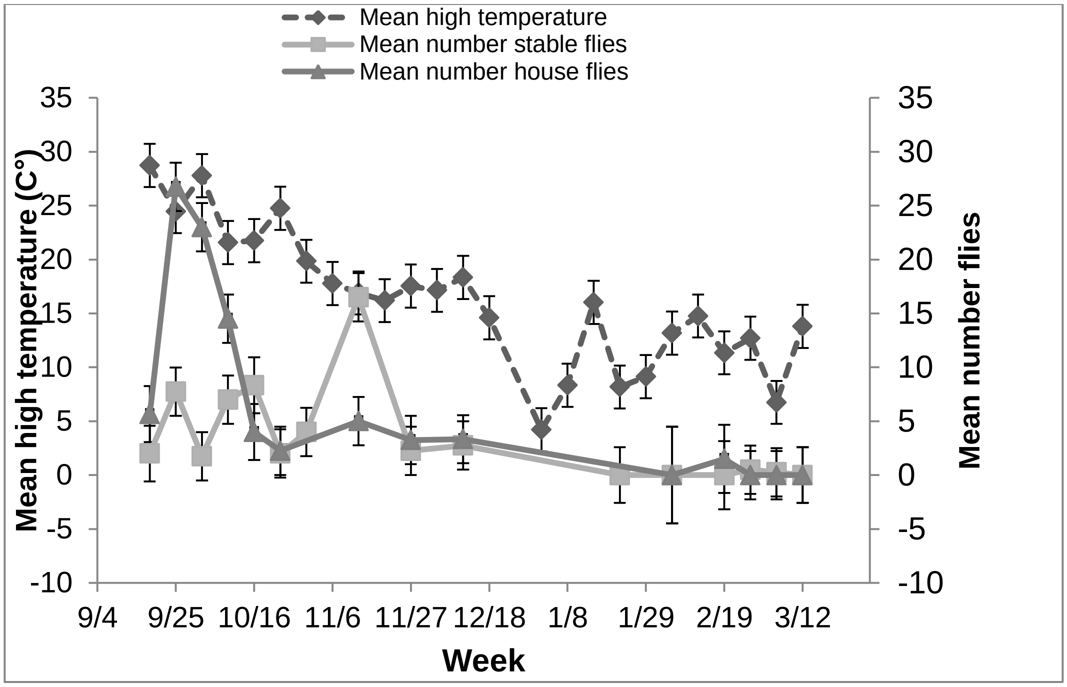


A)

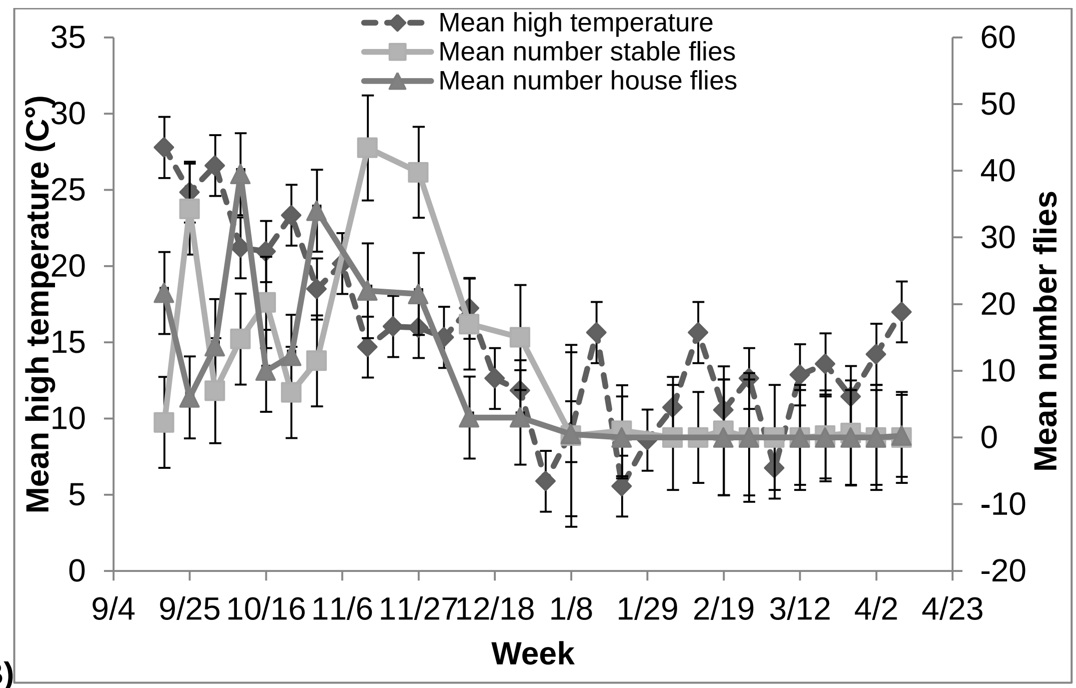


B)

Figure 7. Mean CBG levels of Jersey calves (log transformed) (A) Mean FCI (cortisol/CBG) of Jersey calves (log transformed) (B).



A)



B)

Figure 8. Mean high temperatures and fly populations at MTREC (A) and DREC (B).

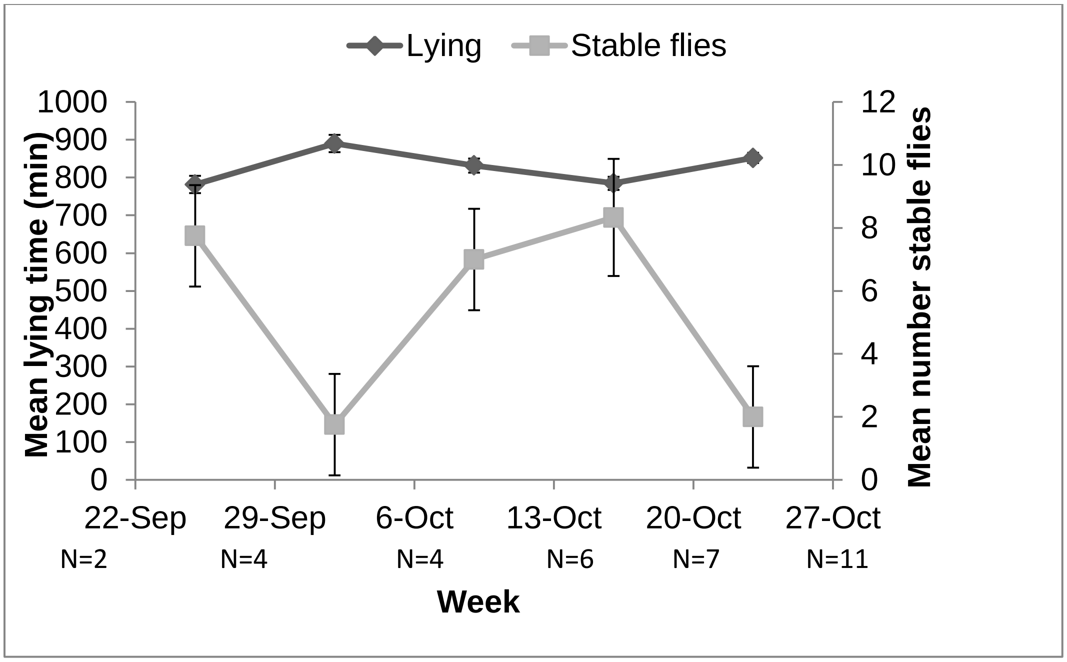


Figure 9. Mean number stable flies compared with mean lying time in Holstein calves P = 0.03; R^2 = 0.82.

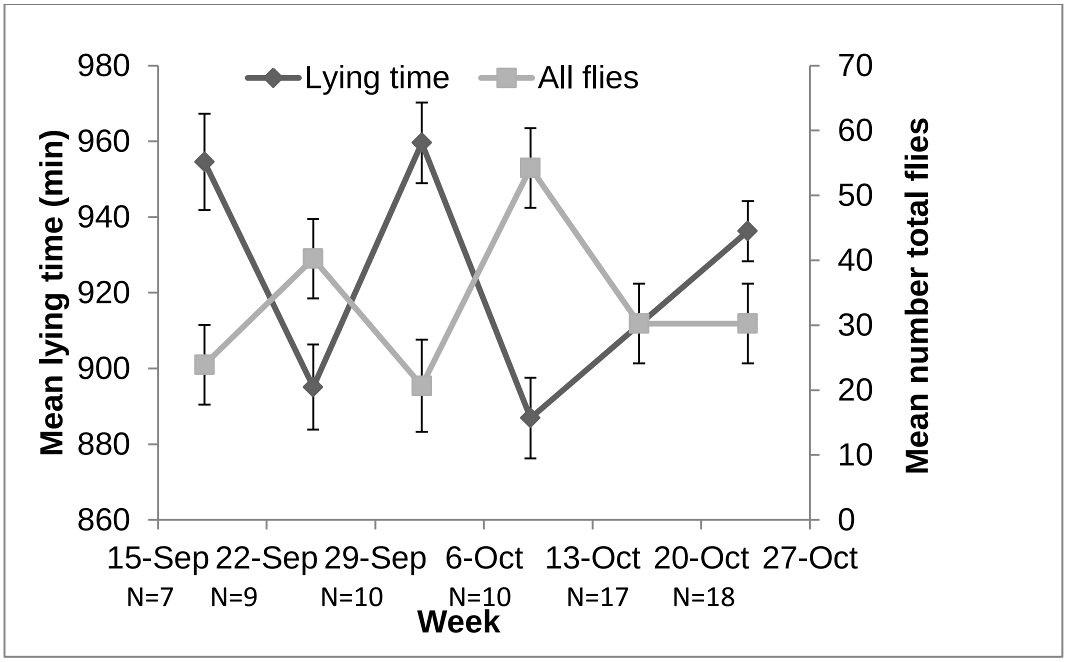


Figure 10. Mean number total flies compared with mean lying time in Jersey calves *P* = 0.03; R^2 = 0.83.

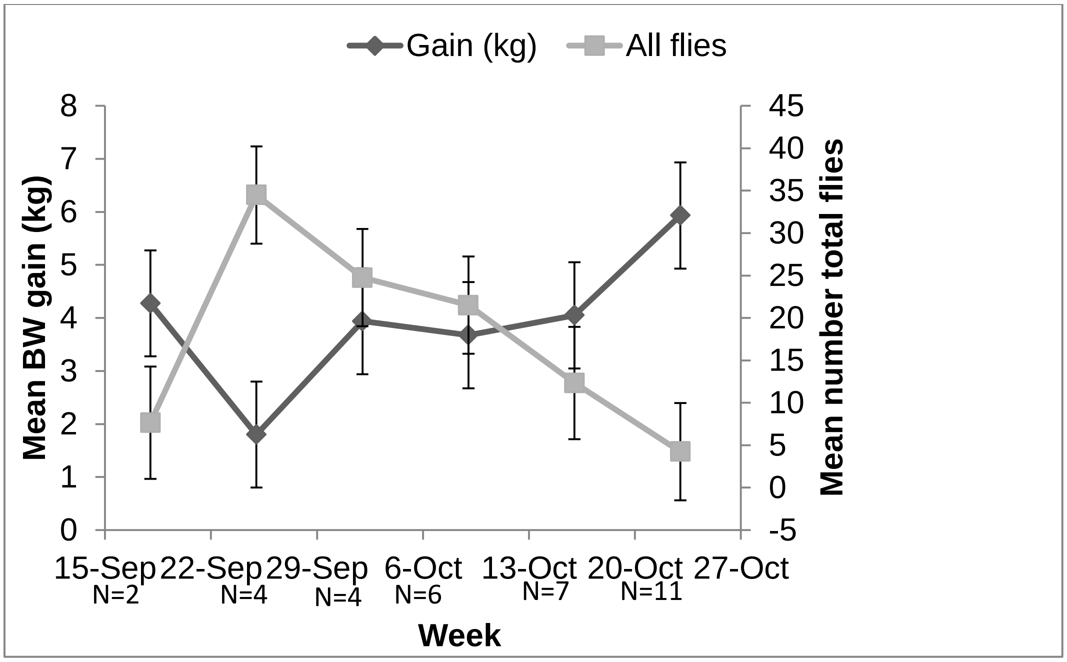


Figure 11. Mean number total flies compared with mean BW gain in Holstein calves P = 0.01; R^2 = 0.81.

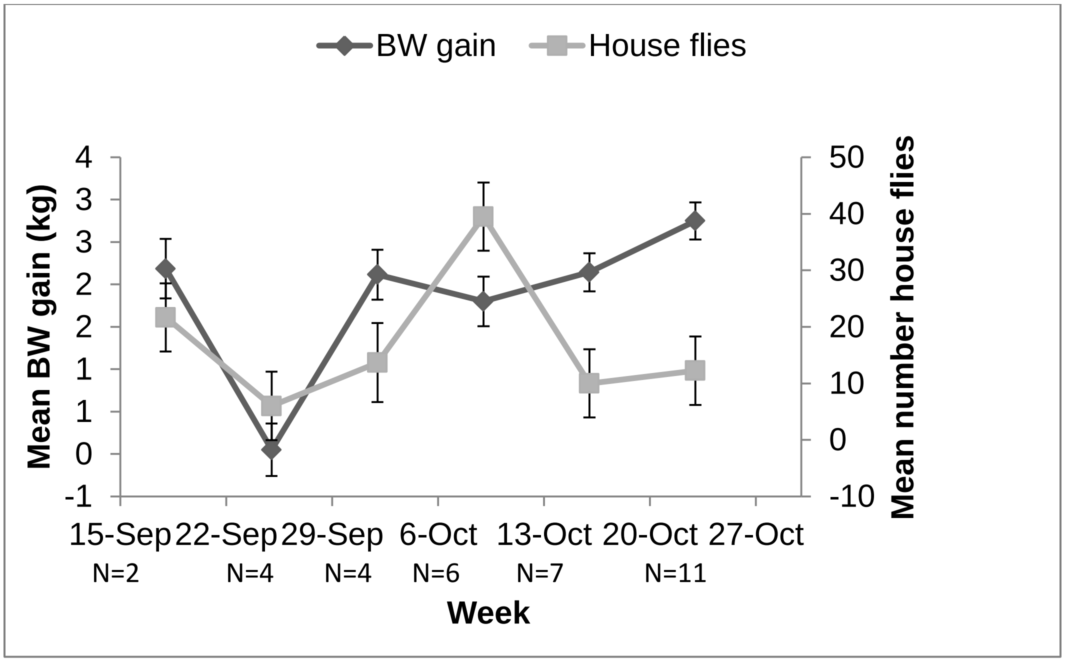


Figure 12. Mean number of house flies compared with mean BW gain in Holstein calves $P = 0.05$; $R^2 = 0.66$.

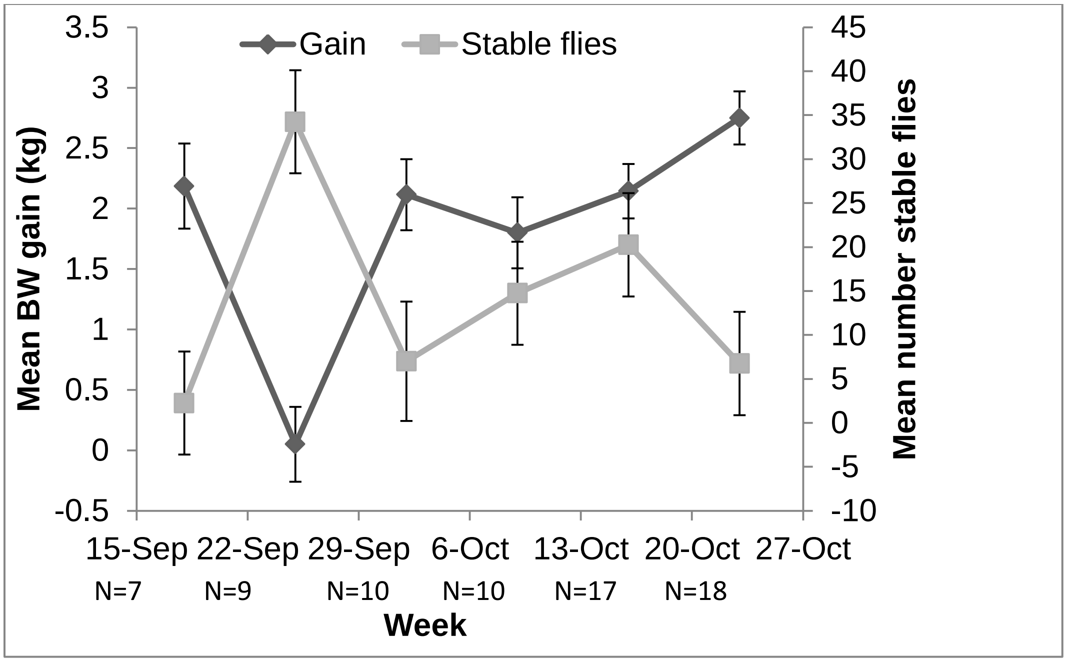


Figure 13. Mean number stable flies compared with mean BW gain in Jersey calves $P = 0.03$; $R^2 = 0.75$.

VITA

Christa Kurman is originally from Southeastern Pennsylvania. She graduated from the University of Delaware in 2012 with a Bachelor’s degree with distinction in animal and food science. Christa also participated in a summer internship at the William H. Miner institute in Chazy, NY. Christa started graduate school at the University of Tennessee in 2012 to obtain a master’s degree in animal science.