DISSERTATION

INFLUENCE OF EARLY LIFE MANAGEMENT STRATEGIES ON INDICATORS OF HEALTH AND PERFORMANCE IN PRE-WEANED DAIRY CALVES AND MATURE COWS

Submitted by

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ABSTRACT

INFLUENCE OF EARLY LIFE MANAGEMENT STRATEGIES ON INDICATORS OF HEALTH AND PERFORMANCE IN PRE-WEANED DAIRY CALVES AND MATURE COWS

Replacement dairy heifers represent the future of dairy operations. Therefore, it is crucial to raise healthy animals that can grow and target reproductive and productive benchmarks throughout their lifetime. Unfortunately, rearing pre-weaned calves is challenging due to their high susceptibility to diseases, in addition to intensive management practices that might lead towards stress.

Four chapters encompass this dissertation: a literature review and three research studies performed in pre-weaned calves and adult cattle. It centers on early management strategies to enhance the health and welfare of pre-weaned calves and the impact of the pre-weaned life in adulthood.

Chapter 1 is divided into four main sections. First, it covers the importance of dairy heifers in production systems, the challenges that pre-weaned calves experience, and how those challenges may affect their optimal health and development. This chapter presents information related to relevant indicators of a successful pre-weaned period, such as increased passive immunity (serum IgG), increased average daily gain (ADG), and decreased morbidity. Secondly, is a review of the scientific literature related to the impact of the pre-weaned period and subsequent productivity. The third section covers probiotics as a management strategy to prevent diarrhea and reduce the

use of antibiotic therapy. Finally, chapter one focuses on the scientific evidence that associates social housing with the welfare and development of pre-weaned calves.

Chapter 2 centers on the carry-over effects of pre-weaned indicators of health and performance in the first lactation of dairy cows. A retrospective study was designed to analyze the association among serum IgG concentration in newborn heifer calves, pre-weaned ADG, and pre-weaned disease events, with fertility, milk yield in the first lactation, and survival during the observed period. In total, the records of 192 cows from four Colorado dairy farms were analyzed (three conventional and one organic). These animals were born over a 12-month period (June 2014 to June 2015), and records were collected in August 2019. Cows were categorized by their serum IgG concentration: Excellent (≥ 25.0g/L), Good (18.0-24.9 g/L), Fair (10.0-17.9 g/L), and Poor (<10.0 g/L); by pre-weaned ADG: Excellent (> 0.82 kg/d), Fair (0.64 to 0.82 kg/d), and Poor (< 0.64 kg/d); and by disease events in the pre-weaned life (0: no, 1:yes). The results of this study did not find an association between age at conception and age at first calving by any pre-weaned parameter. However, the interval calving to conception and age at second calving differed by pre-weaned ADG, favoring the cows in the Excellent category. The pre-weaned indicators were not associated with a difference in the adjusted milk yield at 305 days. The survival of cows from 2014 to 2019 was not affected by the pre-weaned health and performance indicators. However, the survival to the first lactation was associated with disease events in the pre-weaned period.

Chapters 3 and 4 discusses studies where calves were housed in pairs from day 1 of age until weaning. The research presented in Chapter 3 is a randomized clinical trial performed to evaluate the effects of a two-step probiotic program on the health and performance of pre-weaned calves. A total of 236 heifer calves were enrolled at birth and followed until weaning (65±1 d), from July to October 2020. Treatment calves (PB, n=118) were provided 2 probiotic formulations, one in colostrum and a different formulation in the milk. Control calves (CTR, n=118) followed the feeding procedures of the farm and were provided no probiotics. A clinical examination was performed 3 times per week in all the enrolled calves. From a subsample of 50 heifers, 3 fecal samples were collected at 7, 14, and 21 days of life for a complete pathogen screen. In addition, a blood sample was collected at 8 sampling points during the study period for haptoglobin (Hp) quantification. Lastly, body weight was measured at birth and at weaning. The probiotic program did not reduce the incidence of neonatal diarrhea when comparing PB and CTR calves. Furthermore, the probiotic program was not associated with a delay in the onset of neonatal diarrhea, faster recovery from the first diarrhea episode, a greater ADG, or reduced mortality in the study period. Calves in the PB group tended to have a reduced likelihood for *Clostridium spp* in feces, and a reduced concentration of serum Hp at sample points 1 and 5 compared to CTR calves. However, PB calves had a higher concentration of Hp at sample point 2, indicating mild inflammation, compared to CTR calves. This sampling point corresponded to the onset of diarrhea.

Chapter 4 describes an observational study performed with the control calves from Chapter 3 (n=116, 58 pairs). The objective was to describe the behavior of paired housed calves in the first 10 days of housing and the dynamic of diseases within pairs in the first month of life, from July to September 2021. A subsample of 15 pairs was followed for the first 10 days of life to describe the time budget for the following behaviors: resting and active time, play behavior, and time together. Calves were observed one hour before and 1 hour after each milk feeding (AM, Noon, PM), and the temperature-humidity index (THI) was continuously measured inside the hutch. All enrolled calves were clinically examined 3 times per week during the first 30 days of life to evaluate for the presence of diarrhea in each calf. The results indicated that calves increased their active time in the AM and PM period, starting from day 3 in observation; consequently, reducing their resting time. In addition, from day 2 in the AM period, calves began to increase their time together. Similarly, calves started to spend a higher percentage of their active time engaging in play from day 3 of life in the AM and PM periods. Social play and synchronized play behavior increased from day 3 to 6 in the AM period. Contrarily, in the Noon period, no difference was observed for any of the observed behaviors. Furthermore, during the Noon period, calves spent most of their time alone or together inside the hutch. Potentially, due to the environmental conditions, THI was on average 80.6 units in that period. Concerning diseases, at least 1 calf from all pairs was observed with diarrhea in the first 30 days of life. However, in 26% of the pairs, the companion did not develop signs of diarrhea within 5 days. When a paired group of calves presented with diarrhea, the majority were found sick on the same day. When the severity of the health event was analyzed by adding dehydration and attitude status, 52% of the calves developed moderate signs of clinical disease, and 25% of the pair mates developed moderate signs of disease.

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CHAPTER 1: LITERATURE REVIEW

Introduction

Replacement heifers represent the future of dairy operations. Management early in life affects calves optimal health, development, and survival (McGuirk, 2011). The cost of raising replacement heifers, from birth to first calving, has been calculated as $1,808.2 ± 338.6 and £1,819.0 ± 387.0 for the US and UK, respectively (Heinrichs et al., 2013; Boulton et al., 2017). The profitable return to raise calves is not seen until 1.5 lactations (Boulton and al., 2017), and it will largely depend on the age at first calving (Heinrichs et al., 1993). Heifers calving at 23.7 or 25.3 months of age will produce 88% and 82% of milk compared to multiparous cows (Heinrichs et al., 2013). The Dairy Calf and Heifer Association (DCHA) recommendation for age at first calving is 21 to 24 months of life (DCHA, 2016).

The pre-weaned life is characterized by intensive management practices and critical windows of stress (Hulbert and Moisá, 2016). Most of these management practices are inevitable in rearing operations. Calves are separated from the dam immediately after birth, and 86.6% of pre-weaned heifers in the US are raised individually (Urie et al., 2018a). During the pre-weaned period, which lasts an average of 9 weeks (USDA, 2016), calves are exposed to different managements, such as transportation, training for milk feeding, introduction to a solid diet, dehorning, and vaccinations. During the first weeks of life and while all these events are occurring, the calf's immune system is maturing in immunocompetence, and their protection against pathogens relies on passive immunity (McGuirk and Collins, 2004; Hulbert and Moisá, 2016).

Hulbert and Moisá (2016) identified the following critical windows for dairy calves: calving, which is an inflammatory stressor; the second week of life, when calves are highly susceptible to enteric diseases; weaning; and commingling after weaning; with the last two critical windows having been described as a psychological stressor that can lead to an increased susceptibility of respiratory diseases (Hulbert and Moisá, 2016). However, the most critical management factor determining the health and survival of pre-weaned calves is colostrum (Godden et al., 2019). Due to the cotyledonary and synepitheliochorial placenta of ruminants, calves are born agammaglobulinemic and passive immunity is provided through colostrum (McGuirk, 2011; Godden et al., 2019). Failure of passive immunity (FPI) is defined when serum IgG is <10g/L (McGuirk and Collins, 2004). On the other hand, a successful passive transfer of immunity (SPI, ≥10g/L) has been associated with improved health (Lora et al., 2018; Abuelo et al., 2021a) and greater pre-weaned ADG (Elsohaby et al., 2018; Shivley et al., 2018a; Abuelo et al., 2021a).

In the US, a decrease in the incidence of FPT from 19.2% to 12.1% has been observed from 2007 to 2014 (USDA, 2010; Shivley et al., 2018b). Similarly, mortality in pre-weaned calves has decreased from 7.8% to 5% (USDA, 2010; Urie et al., 2018a). However, the recommended survival rate in the pre-weaned life is ≥97% (DCHA, 2016). Therefore, there is still a need for improvement. On the other hand, the morbidity rate has not changed significantly in the last decades (Lombard et al., 2020). The current morbidity for pre-weaned calves has been reported as 33.1% (Urie et al., 2018a). Digestive and respiratory diseases are the most common causes of morbidity in pre-weaned calves, with a reported incidence of 17.2 % and 9.5% and peaks at 2 and 5 weeks of life, respectively (Urie et al., 2018b).

A group of experts proposed a new categorization for serum IgG in young calves, intending to identify if the morbidity of diseases varied by different levels of passive immunity and not only by dichotomization as failure or success (Lombard et al., 2020). This categorization has been widely accepted as the newest recommendation for assessing the transfer of passive immunity in pre-weaned calves in the US. The serum IgG categories and recommended target percentage of calves in herds are Excellent (≥ 25g/L, >40%), Good (18.0-24.9 g/L, 30%), Fair (10.0-17.9 g/L, 20%), and Poor (<10.0 g/L, <10%). Additionally, within this study, it was found that the morbidity of pre-weaned calves in the Excellent versus Poor category was 28.5 vs. 46.1%, and the mortality was 2.5 vs. 7.4% (Lombard et al., 2020). Therefore, this new categorization may allow researchers to explore the effects of the transfer of passive immunity utilizing a more profound approach.

Another important indicator of a successful pre-weaned period, besides the transfer of passive immunity and morbidity, is growth (Chester-Jones et al., 2107). The Dairy Calf and Heifer Association (DCHA, 2016) recommends doubling the birth weight at weaning, that is in average 9 weeks. A national study reported a mean birth weight of 43.2kg, weaning weight of 90.9 kg, and ADG of 0.74kg/day (Shivley et al., 2018a), which is in accordance with the DCHA recommendation. In addition, a group of experts proposed a categorization for ADG in the pre-weaned life (Shivley et al., 2018a). The categories were defined as Excellent (> 0.82 kg/d), Fair (0.64 to 0.82 kg/d), and Poor (< 0.64 kg/d).

Pre-weaned parameters affecting subsequent productivity of dairy cows

The importance of transfer of passive immunity is not under discussion for the pre-weaned life. However, not many studies have addressed if FPI or SPI might impact survival, reproduction, or production in adulthood. Faber et al. (2005) performed a study that assessed the effects of 2 different colostrum feeding programs (2L vs. 4L) in the lactational performance of Brown Swiss cows. The findings of this study did not demonstrate a difference in age at conception, which was 14 months for the two groups (Faber et al., 2005). However, calves fed 4L of colostrum produced significantly more milk. In the first and second lactation, the difference of 305-d mature equivalent

(ME) production was 955kg and 1,642kg, respectively (Faber et al., 2005). In Faber' study passive immunity was not measured, and an assessment of confounders was not included.

Additionally, Pithua et al. (2010) compared two colostrum strategies (plasma-derived colostrum vs. maternal colostrum) in 12 dairy farms. Enrolled calves were followed for 54 months, and the risk of death, culling, lifetime milk yield, and breeding performance was measured. Calves fed plasma-derived colostrum had a higher risk for FPI. Cumulative mortality and culling for the entire study period did not differ by treatment group. However, 18% of the enrolled heifers were lost from birth to first calving in the plasma-derived colostrum group and 5.7% from the maternal colostrum group. No difference was observed for age at first calving, the number of inseminations, calving to conception interval, or milk yield. It is important to remark that this study did not have as an objective the evaluation of passive immunity. Therefore, it is not possible to suggest any conclusion in this respect. However, the information provided is of interest for this literature review. To the best of my knowledge, there is no information available for the categories of serum IgG and any productive or reproductive parameter in adulthood.

There are published studies assessing the impact of ADG in adulthood. Chester-Jones et al. (2017) found that every kg of pre-weaned ADG at 6 and 8 weeks of life was associated with 544±248.8 and 579±241.1 kg more of 305-d ME in the first lactation. Another published study found that every 1kg of ADG in the pre-weaned period was associated with 1,113 kg more of milk in the first lactation (Soberon et al., 2012). In the same study, pre-weaned ADG accounted for 22% of the variation in the first lactation (Soberon et al., 2012). Furthermore, a study performed in the Czech Republic compared the milk yield of cows with pre-weaned ADG ≥0.8 kg/day and ADG ≤0.69 kg/day (Krpálková et al., 2014). Cows in the greater ADG group produced more milk in their lifetime than cows with lower pre-weaned ADG (8,132 vs. 7,110 kg/year; Krpálková et al., 2014). Finally, a mean ADG of 0.8kg/day in the first two months of life was associated with higher survivability to the second lactation when compared to pre-weaned ADG of 0.7 kg/day (Bach, 2011).

Contrasting these results, Gelsinger et al. (2016) in a meta-analysis found that only 2% of the variation in milk yield in the first lactation was explained by pre-weaned ADG, and other management practices were more important for the first lactation performance. Nevertheless, the authors mentioned that the effect of ADG had a more significant influence as ADG increased from 0.5 kg/day to 0.9 kg/day (Gelsinger et al., 2016). Davis Rincker et al. (2011) did not find an association between pre-weaned ADG and 305d-milk yield. Calves in this study were fed 2 different diets, and their mean ADG was 0.44 and 0.64 kg/day, which is suboptimal when compared to other studies.

Potentially, an improved and faster growth might be related to metabolic efficiency, which means that a metabolically efficient calf will continue being efficient as an adult (Sejrsent et al., 2000). Also, a higher plane of nutrition might positively affect the development of the mammary gland (Brown et al., 2005; Gelsinger et al., 2016).

Another topic that has been explored is the impact of pre-weaned diseases on subsequent production as an adult. Abuelo et al. (2021b) retrospectively followed 2,272 cows from a single dairy to assess the influence of diarrhea and bovine respiratory disease (BRD) in reproduction, 305-d ME milk production, and survival to the first lactation. No association was found between diarrhea in the pre-weaned life and the probability of being inseminated, having a pregnancy, or reaching the first lactation. However, these animals required more inseminations and had a reduction in 305 ME of 325kg in the first lactation. On the other hand, cows with at least one pre-weaned BRD event were 14% less likely to be inseminated or reach the first lactation compared to cows with no BRD in the pre-weaned life. No association was found for the number of inseminations or 305ME production.

Moreover, Waltner-Toews et al. (1986) followed cows from 34 farms retrospectively. The results indicated that cows with a history of diarrhea in the pre-weaned life were 2.9 times more likely to calve after 900 days of life and 2.5 times more likely to be sold than cows with no records from diarrhea in the pre-weaned life. Cows with BRD were 2.5 times more likely to die after 90 days of life than cows with no records of BRD. On the contrary, Warnick and White (1995) used the records of pre-weaned diseases of 25 farms and 787 cows. The results indicated no association in the hazard to leave the herds after the first calving by diarrhea or respiratory diseases in the pre-weaned life. The limitation of this study is that only heifers with records of first calving were included. Therefore, it is unknown how many animals were lost before the first lactation and the potential association with disease events in the pre-weaned life.

Neonatal diarrhea and probiotic administration

Diarrhea is the leading cause of morbidity and mortality in pre-weaned calves and one of the most critical problems in dairy calf rearing facilities worldwide (Klein Jöbstl et al., 2014). As mentioned previously, the morbidity of diarrhea in the US was reported as 17.2% (Urie et al., 2018b); the recommendation from the Dairy Calf And Heifer Association is less than 15% of calves experience diarrhea in the pre-weaned period (DCHA, 2016).

Calfhood diarrhea is complex and multifactorial; it can be caused by infectious agents (bacteria, virus, parasites) and non-infectious factors. In the pathogenesis of diarrhea, there are important elements, such as pathogen exposure, environmental conditions, management, nutrition, and immunity (Meganck et al., 2014; Klein Jöbstl et al., 2014). Calves are more susceptible to digestive diseases in the first month of life (Meganck et al., 2014). It has been described that the window for diarrhea starts around the second week of life (Hulbert and Moisá, 2016) when the maternal immunity is declining and when the calf is not immunocompetent (Barrington and Parish, 2001; Smith, 2012).

In the late 80's Curtis et al. (1988) found that a diarrhea episode in the first 14 days of life increased the risk ratio for respiratory disease and death by 2.5 and 2.3 times, respectively. In addition, a reduction in the risk of diarrhea was found when the dam was vaccinated against *E.Coli* (RR=0.2). Donovan et al. (1998) followed calves from birth to 6 months of life; the occurrence and severity of diarrhea were measured as days in treatment. The mean days in treatment were 3.7, and it was associated with a reduction in weight gain of 9.1 kg at 180 days of life. In the same study, diarrhea was associated with a decrease of 5.1% of the pelvic height at 180 days of life (Donovan et al.,1998). Likewise, Abuelo et al. (2021b) found a reduction in pre-weaned ADG of 50 gr/day in calves with a history of diarrhea and antibiotic treatment. However, the impact of pre-weaned diarrhea is not only in the first months of life. As mentioned in the previous section, it has been associated with decreased performance in adulthood.

The economic losses related to diarrhea are due to treatment, decreased performance, and mortality (McGuirk, 2008; Meganck et al., 2015). Furthermore, calf diarrhea has public health implications due to some cases of calf diarrhea are caused by zoonotic disease agents and the potential of antibiotic resistance developing during its use (Frank and Kaneene, 1993). Therefore, prevention of diarrhea should be the goal of any calf health protocol. The base of the program should include peripartum management (vaccines, dam nutrition, environment), calf immunity (colostrum quality, quantity, and timing, passive immunity assessment), environmental stress, and hygiene (Cho et al., 2014).

As the use of antibiotic in food animals is an important concern, the third objective of the USDA Antimicrobial Resistance Action Plan (https://www.usda.gov/sites/default/files/documents/usda-antimicrobial-resistance-action-plan.pdf) is to "identify feasible management practices, alternative to antibiotic use, and other mitigations to reduce antimicrobial resistance associated with food-producing animals and their production environments." Different nutritional strategies have been researched in the last decades to improve the health of pre-weaned calves and reduce the incidence of diarrhea and antibiotic treatments. A few examples are the use of prebiotic (Gosh and Mehla, 2012; Velasquez-Munoz et al., 2019), lactoferrin (Pempek et al., 2019), plant extracts (Salazar et al., 2018; Stefańska et al., 2021), Zinc (Feldman et al., 2019), and probiotics (Timmermann et al., 2005; *Froehlich et al., 2017*; Cantor et al., 2019) have been mixed in milk or calf starter to reduce disease incidence.

One of the most studied nutritional additives is probiotics for the prevention and treatment of neonatal calf diarrhea. These additives may support maintaining an adequate gastrointestinal microbiota, which may lead to improved health, feed efficiency, and performance (Khare et al., 2018) when stressors of intensive production systems might affect the structure of microbial communities (Uyeno et al., 2015). The FAO/WHO defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al., 2014). Uyeno et al.(2015) and Cangiano et al. (2020) agreed that the most common probiotics used for dairy calves are live yeast probiotic (*Saccharomyces cervisiae*), yeast cultures of *Saccharomyces cervisiae,* and single or multi-strain bacterial based probiotic (BBP; *Lactobacillus spp., Enterococcus spp., Bacillus spp*). Uyeno et al. (2015) described the importance of introducing microorganisms that will not disturb the host microbes adapted to the gastrointestinal environment.

The mechanism of action of probiotics is not entirely understood. However, despite its source, this functional food may enhance intestinal health by preventing the colonization of enteric pathogens, boosting the development of beneficial bacteria, improving mucosal immunity, increasing digestive capacity, and lowering the pH (Uyeno et al., 2015). In an invited review, Cangiano et al. (2020) proposed three mechanisms of action for BBP fed to dairy calves: direct interaction with host cells, inhibition of pathogen growth, and modulation of the immune response in the host. Bacterial-based probiotics might increase mucin production in the gut, produce bacteriocins, and through lactic acid production, thereby reducing the pH in the intestine, creating a more favorable environment for commensal bacteria (Khare et al., 2018; Cangiano et al., 2020). Additionally, probiotic bacteria compete for adhesion sites and tighten the epithelial junction to enhance the gut epithelial barrier (Khare et al., 2018; Alawneh et al., 2020; Cangiano et al., 2020). Nonetheless, further investigation towards a better description of the mechanisms is needed.

While numerous studies have tested the effects of probiotics on the growth and health of pre-weaned calves, there is no agreement on whether probiotics benefit calf growth and health. The main effect of probiotics is to reduce the presentation and severity of diarrhea in calves. Timmerman et al. (2005) studied the effect of a calf specific BBP in 4 experiments with veal calves. The results showed a reduction in mild diarrhea (55% vs. 24%) and total diarrhea (65% vs. 33%) when comparing BBP to control calves. In the same experiment, calves fed multi-strain BBP had reduced treatments compared to control calves (Timmerman et al., 2005). A trial using milk replacer supplemented with *Lactobacillus* found a reduction in the morbidity, severity, treatments, and mortality associated with diarrhea caused by Rotavirus in 2 commercial dairy rearing facilities (Kayasaki et al., 2021). Evidence suggests a significant reduction in the recovery from a diarrhea episode when sick calves were administered a multi-strain BBP bolus. Calves treated with BBP bolus recovered on average one day faster than calves not receiving probiotics (5 vs. 6 days; Renaud et al., 2019). Further research is needed to assess the biological significance and the cost associated with this type of treatment.

A meta-analysis described a significant reduction in the pooled estimate for the relative risk of developing diarrhea in calves receiving BBP (RR=0.44; 95%CI=0.25-0.76). No differences were found for fecal consistency in the 12 studies included in the analysis (Signorini et al., 2012). Contrarily, a systematic review found insufficient data to conclude health benefits derived from the supplementation of calves with probiotics (Alawneh et al., 2020). Salazar et al. (2021) found an increase in diarrhea presentation from week 4 to 6 of life in calves fed single-strain probiotic in contrast to calves fed monensin or essential oils. However, when fecal microorganisms were measured, the probiotic bacteria were not detectable. Therefore, the authors concluded that the probiotic formulation was not able to survive the gastrointestinal tract (Salazar et al., 2021).

In relation to performance, Frizzo et al.(2010) found that calves fed with a multi-strain probiotic in milk replacer had an improved ADG in the 35 days of the experiment when compared with the control group (0.70 kg/d vs. 0.38 kg/d). Similarly, when one week old Holstein calves were added a single-strain probiotic (*Bacillus subtilis natto*) in milk replacer, a greater ADG was observed in the pre-weaned life (0.31 vs. 0.35 kg/d). Supplemented calves were weaned at 51d, which was 1 week earlier than control calves (Sun et al., 2010). Cantor et al. (2019) found that calves fed with multi-strain BBP in pasteurized whole milk had a greater ADG in the first 4 weeks of supplementation (0.84 ± 0.10 kg/d vs. 0.74 ± 0.10 kg/d). The supplementation started at week 1 of life, and calves were followed until 1 week after weaning. However, the effect did not persist from week 4 to 8 or after weaning (Cantor et al., 2019). Timmerman et al. (2005) found a statistical difference in ADG in the first 2 weeks after arrival to a rearing veal facility. However, no differences were found in the 8 weeks of study. Moreover, a systematic review found a difference in ADG in calves fed probiotics, the difference was influenced by age, and it was significant only for the first 3 weeks of life (Alawneh et al., 2020). Contrarily, no differences had been reported when providing single-strain probiotic in raw milk (Salazar et al., 2019), in single and multi-strain BBP fed in milk replacer (Zhang et al., 2016), or in a multi-strain BBP fed in milk replacer (Frizzo et al., 2011).

From a meta-analysis (Signorini et al., 2012), a systematic review (Alawneh et al., 2020), and an invited review (Cangiano et al., 2020), there is one major conclusion. The effect of probiotics is noticeable in a setting where calves have a high incidence of pathogenic diarrhea. In addition, the use of multi-strain probiotic provided in pasteurized whole milk seems to be the most effective strategy compared to single-strain probiotic fed in milk replacer (Signorini et al., 2012).

Impact of housing strategies on behavior and welfare of pre-weaned calves

Pre-weaned heifers in the US are predominantly housed individually to prevent disease transmission, monitor feed intake, and prevent agonistic behaviors (USDA, 2016; Urie et al., 2018a; Pempek et al., 2013). In the calf component of the latest USDA/NAHMS national dairy study, it was reported that 13.4% of all calves were housed in groups, which corresponded to 20.2% of the operations (USDA 2016; Urie et al., 2018a).

In the last decades, a substantial amount of research had focused on the benefits of environmental enrichment for dairy cattle. Environmental enrichment includes all the potential modifications in management practices or in the environment of captive animals that will enhance their biological functioning or welfare (Newberry,1995). Mandel et al. (2016) described 5 categories for environmental enrichment in dairy cattle: social, occupational, physical, sensory, and nutritional enrichment. Social or group housing has been one of the most studied forms of environmental enrichment for pre-weaned dairy calves (De Paula Vieira et al., 2012; Costa et al., 2016; Whalin et al., 2018) due to calves being herd animals.

In relation to calf behavior in natural conditions, Vitale et al. (1986) studied semi-wild Maremma calves (*Bos primigenius taurus*) as a parallel for domesticated cattle, due to their behavior had been minimally altered. In this study, calves were followed from birth to 2 months of life. In the first days of life, calves stayed hidden in bushes; however, they were following the dam in the first 3 to 4 days of life. As calves were older than 10 days, cows joined the main herd, whereby the calves started to spend more time farther than 15m from the dam, thereby increasing the time spent with other calves. However, after each milk feeding, the calf and dam spent time grooming. The peak time calves spent with peers was between 11 and 40 days of life. Play activity was higher in the early morning, decreased strongly at mid-day, and increased at mid-afternoon. Therefore, calves in natural and semi-natural conditions are, from an early age, highly sociable.

In production settings, it has been demonstrated that management adjustments must be made to avoid negative effects derived from housing young calves in groups (transmission of diseases and agonistic behaviors, such as competition and cross-sucking; Chua et al., 2002; Svensson and Liberg 2006). For example, when calves are raised in large groups (>10 animals per pen) in contact with calves from other pens, it has been demonstrated an increase of 1.4 (95%CI: 1.21-1.72) times in the odds of respiratory diseases and a reduced ADG of 40g/d, when compared to calves housed in groups smaller than 10 animals (Svensson and Liberg, 2006). Likewise, the results of a study enrolling 1,685 farms in the US found that group housing of 7 or more calves was a risk factor for high mortality when compared to individual calves or calves housed in groups of 6 or fewer animals (Losinger and Heinrichs, 1997).

Additionally, the provision of an adequate milk allowance has been targeted as an essential management practice, mainly to prevent competition and cross-sucking (Chua et al., 2002; Wormsbecher et al., 2017; Whalin et al., 2018). Research analyzing the feeding behavior of calves housed in pairs, from birth to weaning, found a low presentation of cross-sucking in the first 14 weeks of life when calves were offered a maximum of 10L of milk per day (Whalin et al., 2018). Calves were video recorded for 30 minutes after the milk feeding once a week for 7 weeks, and cross sucking was observed 5 times in the experimental period (Whalin et al., 2018). Likewise, a study assessing the behavior and performance of calves housed in pairs found a low frequency of cross-sucking when calves were fed milk ad-libitum (Chua et al., 2002). From the 10 pairs observed, only 3 groups presented the behavior (Chua et al., 2002). In contrast, an increase in cross sucking was described in pair housed calves fed 6L of milk per day (Jensen et al., 2008). Regardless of the treatment group, cross-sucking was observed in the first, second, and third week of life for 40, 50, and 50% of the 24 animals enrolled, respectively (Jensen et al., 2008). Another observed effect of boosting the amount of milk is an increase in play behavior (Krachun et al., 2010; Jensen et al., 2015). Play behavior is an indicator of good welfare, and it is performed when animals have their basic needs covered (Jensen et al., 1998; Krachun et al., 2010).

With appropriate management practices, an increase in calf starter consumption and greater weight gains had been reported in calves housed with a companion (De Paula Vieira et al., 2010; Knauer et al., 2020). Furthermore, important welfare benefits were associated with social housing, such as developing social skills, improved cognition, and response to novelty (Costa et al., 2016). Moreover, some of these benefits had been demonstrated to have an impact beyond the pre-weaned stage (Knauer et al., 2020).

In relation to social behavior, calves that are housed individually are less active than calves in social housing (Jensen et al., 1998). Different types of play behavior have been described for dairy calves housed individually or with a companion. Locomotor play refers to a single calf engaging in galloping, bucking, kicking, body, and head rotations (Jensen et al., 1998; Jensen et al., 2015). Contrarily, social play consists of 2 or more calves performing locomotor play directed towards another calf, and the interaction does not result in fight or submission (Jensen et al., 1998; Jensen et al., 2015). Lastly, locomotor synchronized play behavior is referred to when 2 or more calves perform locomotor play at the same time, without interaction (Pempek et al., 2016). With these concepts in mind, socially housed calves have the advantage of expressing social behavior with conspecifics. When the behavior of pair and individually housed calves was recorded at 3 times points in the pre-weaned period, the results showed that individually housed calves performed locomotor play for a longer duration than paired housed calves (Jensen et al., 2015). However, paired housed calves engage consistently in social play behavior (Jensen et al., 2015). Duve and Jensen (2012) followed calves from birth to 6 weeks of life. Calves were housed individually, in pairs since birth, or in pairs after 3 weeks of life. Calves with full social access performed more play behavior than calves housed individually. Additionally, calves housed together since birth started to sniff and lick their companion from day 2, suggesting that calves will interact with each other early in life. However, calves were only recorded on days 2, 12, 22, and 34 of life (Duve and Jensen, 2012).

A study compared the bonding of calves raised in pairs since birth or since week 3 of life with the behavior of calves housed individually with limited contact to conspecifics through bars (Duve and Jensen, 2011). Calves were tested starting at 5 weeks of life in a preference test. The results demonstrated that calves with full contact approached the familiar calf first. In contrast, calves with limited contact approached the unfamiliar calf first. And when placed with an unfamiliar calf, full contact calves spent less time pushing and mounting than calves with limited contact. Although, they spent then the same amount of time licking and sniffing. These results suggest that when calves are raised with full contact, they establish a stronger bond than calves raised with limited contact. Likewise, it has been suggested that calves are willing to "work" to have full contact with another calf. Holm et al. (2002) performed a conditioning study to assess calves ' motivation for 2 types of social contact: full contact or head contact through metal bars. Twelve calves were enrolled in this study and trained to press a panel to open a gate that gave them access to the companion calf for 3 minutes. Calves were motivated to work more to have full contact with the companion calf. In addition, calves were socially active for 8.3% and 54.1% of the time when they had partial or full contact, respectively. This study suggests that full contact with conspecific has a high value for calves, which allows them to express their playful nature. This is valuable information and needs to be taken into consideration for welfare purposes.

Gaillard et al.(2014) analyzed the cognition of calves in 2 tests comparing individual versus pair housed calves. The first test was a reversal learning, and the second one the reaction to a novel object. Calves were trained to discriminate between 2 colors to obtain a milk reward. Individual and paired housed calves had a similar rate of learning. However, when the task was reversed, and the milk reward was associated with the unrewarded color, paired housed calves had a better performance in the test than individually housed calves. Later, calves were exposed to a novel object, a red plastic bin for 8 times in 2 days. Paired housed calves showed less exploration with the repetition; however, individually housed calves did not change their exploratory behavior. In addition, Costa et al. (2014) measured the response of calves to a novel food. Calves were housed individually or in a complex social group (dam and other calves).Calves were exposed to 2 buckets, one empty and the other filled with novel food. Socially housed calves had a shorter latency to eat the food and consumed more in the 30 minutes per 3 days that the tests lasted (35+-6 g/d 18+-6g/d). Furthermore, it has been reported that calves raised in pairs vocalized less at weaning, had a shorter latency to consume calf starter, stay for longer periods at the feeder, and consumed more starter than calves individually housed (De Paula Vieira et al., 2010).

These results suggest that calves housed in pairs or small groups had a better transition or adaptation to changes, were less fearful, and had a better development of their social skills. This may significantly impact how calves respond to weaning, commingling, or other management practices later in life. Moreover, no negative effects have been reported when management strategies adapt to the needs of milk and space.

Final remarks

There is an increasing interest and body of knowledge around dairy calf health, behavior, and performance. In addition, considerable improvements have been made in the last decades to enhance dairy calf welfare. However, there are still unknowns or insufficient scientific evidence on the relation of pre-weaned management practices and their influence in adulthood, in the effects BBP on the health of calves, and in the daily behavior of young calves as an adaptation to social housing or in their disease dynamics. Therefore, the objective of this dissertation is to provide scientific information concerning these topics.

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CHAPTER 2: ASSOCIATION AMONG PRE-WEANED INDICATORS OF IMMUNITY, HEALTH, AND PERFORMANCE WITH FERTILITY, SURVIVAL, AND MILK YIELD OF FIRST PARITY COWS

Introduction

Raising dairy heifers represent 15 to 20% of the total production cost of dairy operations (Heinrichs et al., 1993). Therefore, maintaining optimal performance of replacement calves is critical to reaching adequate reproductive and productive targets (Abuelo et al., 2021a). In addition, identifying early indicators of optimal future milk production and longevity could assist in recovering the investment of the rearing period (Van De Stroet, et al., 2016). Some critical indicators of health and performance in pre-weaned dairy calves are passive immune transfer, ADG, and disease occurrence. However, there are still unknowns related to the carry-over effects of the pre-weaned life in adulthood and the survival of cows through the first lactation.

Passive immunity through colostrum feeding has been identified as the most critical management in the early life of dairy calves (Godden et al., 2019). A new categorization for IgG and serum total protein has been recommended to assess passive immunity in dairy operations (Lombard et al., 2020). However, despite the importance of passive immunity early in life, little is known on how this parameter can affect reproduction, survival past the first lactation, or milk yield later in life. A recent study evaluated the impact of 1 or 2 colostrum feedings on age to puberty and to first calving (Abuelo et al., 2021b). Although calves fed 2 colostrum feedings had lower odds for failure in passive transfer, for bovine respiratory disease (BRD), and a greater ADG in the pre-weaned life, no association was found on age to puberty or calving (Abuelo et al., 2021b). Additionally, one study following calves from birth to 52 months of age did not find an association between the type of colostrum fed (maternal vs. plasma-derived) and the risk of death or culling during the 52 months of study, The same study showed that calves fed plasma-derived colostrum were more likely to have a failure of passive immunity (Pithua et al., 2010).

Digestive and respiratory diseases are the two leading causes of morbidity and mortality in the pre-weaned dairy calves. A national study reported that the incidence of diarrhea and respiratory diseases were 17.2% and 9.5%, respectively (Urie et al., 2018a). The consequences of diseases in the pre-weaned life are associated with higher costs due to treatments, reduced weight gain, and loss of animals (Meganck et al., 2014; Klein Jöbstl et al., 2014). Waltner-Toews et al. (1996) reported that heifers treated for pneumonia before weaning were 2.5 times more likely to die after 90 days of age than heifers not treated for pneumonia, and heifers treated for diarrhea were 2.5 times more likely to be sold before the first lactation and 2.9 times more likely to calve after 900 days of life than heifers not treated for diarrhea. Recently, Abuelo et al. (2021a) evaluated the effect of pre-weaned diseases in reproductive performance and milk production in the first lactation. Interestingly, pre-weaned heifers that experienced diarrhea subsequently required more inseminations to become pregnant, and they produced 325 kg less of 305 days adjusted milk in the first lactation. However, no association with diarrhea was found for entering first lactation. On the other hand, heifers with BRD before weaning were less likely to be inseminated or reach the first lactation than heifers with no records for BRD. Although, the cows that made it to the first lactation were not different for the age at first insemination or adjusted milk yield compared to the cows that did not present BRD in the pre-weaning (Abuelo et al., 2021).

There is more information related to the effect of pre-weaned growth and ADG on milk production in the first lactation, especially when comparing different nutritional plans (Davis Rincker et al., 2011; Korst et al., 2017). Cows with better pre-weaned growth and ADG produced between 291 and 765kg more of milk (Davis Rincker et al., 2011; Korst et al., 2017). In addition, pre-weaned heifers with ADG equal or greater than 0.82kg/d produced 1,120 kg more milk in the first lactation than heifers with an ADG of 0.55 kg/day (Hayes et al., 2021). Similarly, it has been described that for every kg increased of pre-weaned ADG, cows produce 850 and 1,550 kg more of milk in the first lactation (Soberon et al., 2012; Soberon and Van Amburgh, 2013). Despite these positive results, a meta-analysis concluded that other management practices were more important in determining milk yield in the first lactation than pre-weaned ADG (Gelsinger et al., 2016). Although, the authors highlighted that keeping ADG above 0.5 kg/d improved the performance of the first lactation cows.

There is scientific evidence to consider that pre-weaned indicators of health and performance might impact future reproduction and production in adulthood. However, more information is still needed. Therefore, the objective of this retrospective study was to evaluate the carry-over effects of passive immunity considering serum IgG category, pre-weaned health, and pre-weaned ADG on reproductive, productive, and survival parameters to the end of the first lactation. We hypothesized that cows with excellent passive immunity (≥ 25g/L serum IgG), with no diseases, and with ADG over 0.82kg/d would perform better than cows with poor passive immunity (<10.0 g/L serum IgG), with at least one health event in the pre-weaned life, and with ADG lower than 0.64 kg/

Material and methods

Experimental design, farms, and animals

This trial is an observational and retrospective study that had as objective to explore the carry-over effects of pre-weaned indicators of health and performance into adulthood. In total, four large commercial dairy farms from Northern Colorado were recruited.

The dairy farms and the dairy cows included in this study represented Colorado in the calf component of the NAHMS Dairy 2014 study. Therefore, this was a convenience sample size of 192 Holstein heifers born between June 19th, 2014, and June 8th, 2015. Three of the operations were conventional dairy farms, and one was a certified organic dairy farm.

Data Collection

Data were collected at 2 time periods, prospectively in the pre-weaned life and retrospectively from weaning to adult life.

USDA researchers facilitated data related to pre-weaned characteristics of the enrolled cows. The dataset included farm ID, calf ID, dam ID, date of birth, calf breed. In addition, serum IgG category, ADG for the pre-weaned period, and a binary variable for disease occurrence in the pre-weaned life were available for each calf in the data set. Details about data collection and descriptive characteristics of pre-weaned heifers were published by Urie et al. (2018b).

Serum IgG concentration was categorized according to the latest recommendations for passive immune transfer (Lombard et al., 2020) as Excellent (≥ 25g/L), Good (18.0-24.9 g/L), Fair (10.0-17.9 g/L), and Poor (<10.0 g/L). The information related to the sampling procedures and analyses was described by Shivley et al. (2018a). Briefly, blood samples were collected from animals between 1 and 7 days of life, and radial immunodiffusion was used to measure serum IgG concentration.

Disease events during the pre-weaned life were summarized as a binary outcome. Consequently, animals were classified into two groups, which presented at least one health event and those that did not present a health event in the pre-weaned life. Producers participating in the calf component of the NAHMS Dairy 2014 study were provided the Calf Health Scoring Chart

(McGuirk. 2008) to report clinical signs. Detailed information related to the collection of health data was published by Urie et al. (2018b). Notably, 83% of the enrolled animals that had at least one health event required a treatment intervention, as established in farms' SOPs. Therefore, disease in this data set corresponded largely to treatments.

Average daily gain from birth to weaning was available for 166 of the 192 enrolled animals. Most of the animals missing pre-weaned ADG were animals that died prior to weaning. For the calculation of ADG, birth and final weights were measured using the heart girth circumference tape from Coburn (Nasco, Fort Atkinson, WI). Birth weight was subtracted from the final weight and divided by the number of days between birth and final weight. As indicated by the authors, not all final weights corresponded to weaning. However, only calves with the final weight collected within 14 days of weaning were considered. For this trial, ADG was categorized following the description of Shivley et al. (2018b). ADG was considered Excellent (> 0.82 kg/d), Fair (0.64 to 0.82 kg/d), and Poor (< 0.64 kg/d). Detailed information related to pre-weaned ADG was published by Shivley et al. (2018b).

For the adult data set, animal records were collected in August 2019. The data management software used by the farms were PCDart (DRMS, Raleigh, NC), DairyComp 305 (VAS, Tulare, CA), and DHI-Plus (Amelicor, Provo, UT). Visits to the farms were scheduled to obtain data file backups and to obtain specific codes for the input of the data.

Each cow enrolled in this trial was searched by ID number in the record system. The identity was confirmed by the date of birth and dam ID. When available, lifetime records from weaning to August 2019 were extracted from farm software and organized in a Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheet. A summary for cow survival was created, including the status of the cow in the herd (active or culled), date and cause of culling or death, age at culling or death, and lactation number when the culling or death occurred. When heifers left the herd before the first calving, they were considered culled at lactation zero.

For the current study, information regarding reproduction and production was used and organized as follows. From weaning to first calving, the variables collected were the last breeding date, age at conception (days), and pregnancy confirmation. From each lactation, the variables collected- when available- were the date of calving, age at calving (days), date of the last breeding, days at conception, total milk yield for lactation (kg), days in milk (DIM), dry period length (d). In addition, daily milk yield (kg/d) and adjusted milk yield to 305 days (kg) were calculated for each lactation and added to the spreadsheet.

The pre-weaned calf data, provided by USDA researchers, was merged with the adult data set by ID number and date of birth. In addition, the season of birth (Summer= June, July, August; Fall= September, October, November; Winter=December, January, February; Spring= March, April, May) was added to the data set.

Statistical Analysis

All the data were organized on Microsoft Excel (Microsoft Corporation, Redmond, WA), and statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., Cary, NC). Significance level was considered at P-value <0.05

Descriptive statistics were obtained with PROC FREQ for the distribution of animals by farm, year of birth, the season of birth, IgG category, pre-weaned ADG category, and pre-weaned health events. In addition, PROC FREQ was used to determine the proportions of animals that started the first and second lactation. In addition, PROC MEANS was used to determine mean values for productive (milk yield, adjusted milk yield, DIM) and reproductive (days at conception, age at first and second calving) characteristics for all eligible cows.

All the outcomes of interest were analyzed with mixed models. Continuous outcomes, such as age at conception, age at first and second calving, milk yield first lactation (total, adjusted to 305 days and daily), interval calving to conception, DIM in the first lactation were analyzed with a general linear mixed model using PROC MIXED. The three main predictor variables were serum IgG category, pre-weaned ADG category, and pre-weaned health status. All predictor variables were evaluated independently. The models were adjusted by season and year of birth as fixed effects, and farm ID was used as a random effect.

Binary outcomes, such as animals that left the herd at different stages (before the first calving, in the first lactation, or in the entire period observed), were analyzed with multiple logistic regression using PROC GLIMMIX. The predictors of interest were adjusted by season and year of birth, and farm ID was used as a random effect. In addition, PROC LIFETEST and PROC PHREG were used to determine the survival curve and the hazard ratio for the cows that left the herd before the first lactation, in the first lactation, or for the complete period observed by the predictors of interest. The Cox proportional models were adjusted for season and year of birth and farm as a random effect. Censoring was conducted at 1,900 days.

Results

Overall, 64 cows were active in their respective herds in August 2019. The only completed lactation was the first lactation, and information for the beginning of the second lactation was available for all surviving cows. For this reason, the current trial focused on productive and reproductive parameters only for the first lactation. In terms of survival, cows were considered from birth to August 2019.

Descriptive statistics

A total of 192 calves born between June 19th, 2014, and June 8th, 2015, were included in this observational study. Four farms were enrolled for this trial; the distribution of calves enrolled per farm is shown in Table 2.1. The number of animals per farm ranged from 34 to 62, representing 17.7% and 32.3% of the enrolled cows.

Overall, 55.7 % (n=107) of the cows were born in 2014 and 44.3% (n=85) in 2015 (Table 2.1). Furthermore, 35.9% (n=69) of the cows were born in summer, 27.1% (n=52) were born in spring, 21.9% (n=42) were born in winter, and 15.1% (n=29) was born in fall (Table 2.1).

The pre-weaned characteristics for the calves enrolled in this study are shown in Table 2.1. When IgG concentration was categorized, 40.6% (n=78) of the enrolled animals were in the Excellent category, 29.7% (n=57) were in the Good category, 18.8% (n=36) were in the Fair category, and 10.9% (n=21) were in the Poor category. Regarding ADG, 42.8% (n=71) of the animals were in the Excellent category, 30.7% (n=51) were in the Fair category, and 26.5% (n=44) in the Poor category (Table 2.1). The ADG for the pre-weaned life was not available for 26 of the enrolled calves; most animals missing the ADG record died before weaning. Concerning disease occurrence in the pre-weaned life, 67.2% (n=129) of the enrolled calves did not have health events, and 32.8% (n=63) had at least one health event (Table 2.1).

In total, 46 heifers died or were culled before the first calving. Therefore, 146 (76%) heifers reached the first lactation. However, 108 (56.3%) cows completed the first lactation and started a second lactation. Detailed information regarding the number of cows that had the first and second lactation by each level of the predictor variables can be found in the Appendix section (A2.1). The distribution by IgG category of cows entering first lactation was 44.5% (n=65) in the Excellent category, 28.8% (n=42) in the Good category, 17.1% (n=25) in the Fair category, and 9.6% (n=14) in the Poor category. The distribution of heifers by pre-weaned ADG was 35.5% (n=59) were in the Excellent category, 25.9% (n=43) in the Fair category, and 21.1% (n=35) in the Poor category. Finally, the distribution of heifers by pre-weaned disease was 56.2% (n=108) for heifers with no disease events, and 19.8% (n=38) for heifers that presented at least one health event.

For the group of cows that had at least one calving, the mean (±SE) age at conception was 447.7 (±5.17) days, the mean (±SE) age at first calving was 725.6 (±5.17) days, the mean (±SE) DIM in the first lactation was 328.2 (±10.1) days, the mean (±SE) milk yield for the first lactation was 10,751 (±384.1) kg, and the mean (±SE) adjusted production to 305 days was 9,660 (±218.2) kg.

From the 108 cows that reached the second lactation, 48.1% (n=52) was from the Excellent category, 26.9% (n=29) from the Good, 14.8% (n=16) from the Fair, and 10.2% (n=11) from the Poor category for IgG. In relation to ADG category, 42.4% (n=42) were from the excellent, 30.3% (n=30) from the Fair, and 27.3% (n=27) from the Poor category. In addition, 74.1% (n=80) did not experienced disease in the pre-weaned life, and 25.9% (n=28) experienced at least one disease event.

Age at conception and age at calving

No differences were observed for the mean age at conception as heifer for ADG category (P=0.59; Table 2.2) or pre-weaned disease (P=0.91; Table 2.2). A trend was found for age at conception (LSM±SE) by IgG category (P=0.05; Table 2.2), where heifers in the Excellent and

Poor category conceived at 14 months (and 446.3±25.9 and 439.2±7.8 days of life, respectively) in comparison with heifers in the Fair category that conceived at 15 months (468.9±25.6 days of life). Similarly, no difference was determined for age at first calving by ADG category (P=0.59, Table 2.2) or disease (P=0.91; Table 2.2) in the pre-weaned life. A trend (P=0.05; Table 2.2) was found for the mean (LSM±SE) age at first calving by IgG category. Cows in the Excellent (724.3±25.9 d) and Poor (717.2±27.8 d) category calved earlier than cows in the Fair category (746.9±26.6 d).

In relation to the calving to conception interval, no differences were found for IgG category (P=0.21; Table 2.2) and disease presentation in pre-weaned life (P=0.56; Table 2.2). A significant difference was found for the ADG category (P=0.03; Table 2.2). Cows that had an Excellent pre-weaned ADG had a mean (±SE) conception after the first calving of 99.7 (±16.6) days in comparison to cows in the Fair and Poor category that had a mean (±SE) conception at 148.5 (±19.1) and 59.6 (±20.4) days, respectively. Similarly, age at calving for the second lactation was statistically significant for ADG category in the pre-weaned life (P=0.02; Table 2.2). Cows in the Excellent category calved at a mean (±SE) age of 1,109 (±34.4) days in comparison to cows in the Fair and Poor category that calved at 1,161 (±35.9) and 1,179 (±36.8) days of life, respectively. No differences were found for age at second calving by IgG category (P=0.17; Table 2.2) or disease in the pre-weaned life (P=0.74; Table 2.2).

Days in milk in the first lactation

Of the 146 cows that reached first lactation, the mean DIM did not differ by IgG category (P=0.98; Table 2.2), disease events in the pre-weaned life (P=0.38; Table 2.2), or ADG in the pre-weaned life (P=0.11; Table 2.2). However, it is important to mention that 51.4% (n=75) of the cows that achieved the first lactation had over 305 days in milk (range: 309-742 days). In addition, 7 cows had less than 100 DIM in the first lactation (range: 6-94 days). Finally, 64 cows had a lactation between 105 and 305 days.

Milk yield in the first lactation

No differences were detected for the total milk yield by IgG category (P=0.95; Table 2.3), ADG category (P=0.64; Table 2.3), or disease presentation in the pre-weaned life (P=0.39; Table 2.3). Likewise, the adjusted milk yield to 305 days was not different by IgG category (P=0.65, Table 2.3), ADG category (P=0.49; Table 2.3), or disease presentation in the pre-weaned life (P=0.12, Table 2.3). Furthermore, the daily production for the lactation did not differ by IgG category (P=0.65; Table 2.3), ADG category (0.12; Table 2.3), or disease in the pre-weaned life (P=0.49; Table 2.3).

An additional analysis was performed considering the milk that was not produced by the heifers that left the herd before the first lactation. The total milk yield, the adjusted milk to 305 days, and the daily milk yield were calculated and analyzed. The only variable that was statistically significant was disease presentation during the pre-weaned life (Table 2.4). For total milk yield, cows that experienced any disease as a calf produced 1,892 kg less than cows that did not experience any disease (P=0.04; Table 2.4). When milk was adjusted at 305 days, cows that experienced at least one disease event in the pre-weaned life produced 1,946 kg less than cows that did not experience any disease (P=0.007; Table 2.4). Finally, the daily milk production was on average 6.5 kg less for cows that experienced at least one disease in the pre-weaned life than those that did not present a disease event (P=0.006; Table 2.4). No differences were found for milk production by IgG and ADG category. However, the adjusted and daily milk yield presented numerical differences when comparing the Excellent versus the Poor category of each variable

(Table 2.4). When comparing the previously mentioned categories, the difference in production was 1,532 kg and 1,120 kg for serum IgG and pre-weaned ADG, respectively.

Leaving the herd

From the enrolled animals, 23.9% (n=46) of the heifers left the herds between birth and the first calving. Causes for leaving the herds were deaths (n=28; 60.8%), sold for reproductive problems (n=10; 21.8%), and sold to another dairy (n=8; 17.4%). No differences were found in the odds of a heifer for leaving the herds before first calving for IgG category (P=0.33; Table 2.5) or pre-weaned ADG category (P=0.77; Table 2.5). Contrarily, a significant difference was found for disease presentation during the pre-weaned life (P=0.001). Heifers with at least one health event in the pre-weaned life were 3.3 times more likely to leave the herd than the heifers with no health event as pre-weaned calves (Table 2.5). Similarly, heifers that presented a pre-weaned disease event had a hazard to leave the herds of 2.83 times compared to heifers that did not present a disease event in the pre-weaned life (P=0.0005; Table 2.6; Figure 2.1)

During the first lactation, 26.0% (n=38) of the cows left the herds. Causes for leaving the herd during the first lactation were deaths (n=1; 2.6%), sold for low production (n=3; 7.9%), sold to another facility (n=5; 13.2%), sold for mastitis (n=6, 15.8%), sold for reproductive problems (n=17; 44.7%), and sold due to diseases other than mastitis and reproductive problems (n=6; 15.8%). No differences were found in the odds of a cow for leaving the herds during the first lactation for IgG category (P=0.48; Table 2.5), pre-weaned ADG category (P=0.79; Table 2.5), or pre-weaned disease status(P=0.82; Table 2.5). Likewise, no differences were found in the hazard to leave the facilities for the 3 main predictors (Table 2.6).

For the entire period (2014 to 2019), a total of 130 (67.7%) cows left the herds. The causes for leaving the herds were sold for reproductive problems or abortions (n=16; 34.8%), sold due to mastitis (n=9; 19.5%), sold for digestive reasons (n=7; 15.2%), deaths (n=5; 10.9%), sold for unknown reasons (n=5; 10.9%), sold for production (n=2; 4.3%) , sold for lameness (n=1; 2.2%), and sold to another dairy (n=1; 2.2%). No differences were observed in the odds of a cow for leaving the herds from 2014 to 2019 for ADG category (P=0.69; Table 2.5) and pre-weaned disease status (P=0.15; Table 2.5). A trend was found for the odds of leaving the herd from 2014 to 2019 when comparing the IgG categories Excellent, Good, and Fair vs the Poor category (P=0.05; Table 2.5). Cows in the Excellent IgG category had a reduced hazard of leaving the herds from 2014 to 2019 compared to cows in the Poor category (HR= 0.67, 95%CI: 0.33-0.88, P=0.04; Table 2.6; Figure 2.2). In contrast, the hazards for leaving the herds by ADG category and disease in the pre-weaned life were not statistically significant (Table 2.6)

Discussion

This objective of this study was to evaluate the carry-over effects of pre-weaned passive immunity, health, and performance on adult reproduction, production, and survival.

Age at conception and age at calving

Age at first conception is an important fertility benchmark in dairy cattle (Novakovic et al., 2011) because it sets the age at first calving. The gold standards set by the Dairy Calf and Heifer Association (2016) recommend starting the breeding of Holstein heifers between 12 to 13 months of age at 55% of the mature body weight. Additionally, the recommendation for first calving is between 21 to 24 months of life at 85% of mature body weight and a BCS of 3.25 to 3.50 (DCHA, 2016).

In this study, no difference was found for the age at conception for any of the pre-weaned parameters analyzed. However, this group of heifers had a mean conception past the 14 months of life (439 to 468 days). Although this was not ideal when considering the previously mentioned recommendations, there are studies describing age at first conception over 491.19 ± 9.36 days of life in high producing dairy cows (Novakovic et al., 2011).

Consistent with our results, when a group of cows was followed from birth to conception with 2 different housing and milk allowance managements (group vs. individual; ad-libitum vs. restricted), no differences were found in their age at conception; even though the group fed ad-libitum had greater growth and BCS (Curtis et al., 2018). In the same study, the presentation of diseases in the pre-weaned life did not affect the age at first conception, which was on average 67.7 weeks of life (473 days of life; Curtis et al., 2018). In Brown Swiss cows, a study provided 2 or 4 liters of colostrum after birth; no differences between groups were found for the age at first conception (<14 months of life; Faber et al., 2005). However, in this study, passive immunity was not measured; therefore, there is a significant limitation when comparing our results.

Contrasting these results, heifers that had an improved ADG in the pre-weaned life, due to a high-energy and high protein diet, had a conception of 15 days earlier (423 days of life) than calves fed a standard milk replacer diet (Davis Rincker et al., 2011). In addition, a study evaluating the impact of the first 14 weeks of life in the lifetime of the cows found that the number of days with of diarrhea or coughing had a negative effect on age at first calving (Heinrichs and Heinrichs, 2011).

The cows in our study calved between 717 and 747 days of life when considering all pre-weaned parameters analyzed. No differences were found for age at first calving by ADG and disease in the pre-weaned life, although a trend was found for IgG category. Interestingly cows in the Poor category calved 7 days earlier than the Excellent category and 29 days earlier than the Fair category cows; this was an unexpected finding.

In accordance with our results, no differences in age at first calving (745 vs. 775 days of life) were found when heifers fed ad-libitum milk had greater ADG than heifers fed restricted milk (Korst et al., 2017). Similarly, no differences in ADG were found in calves fed maternal or plasma-derived colostrum after birth, even though plasma-derived calves had a greater likelihood for failure in passive transfer (Pithua et al., 2010). Contrarily, cows with a higher ADG during the pre-weaned life calved on average 14 days (701 days of life) earlier than calves with lower ADG in the pre-weaned life (Davis Rincker et al., 2011). Additionally, a study found a trend for a greater number of days in treatment and an increase in age at first calving when considering health events in the first 4 months of life (Heinrichs et al., 2005).

Keeping an optimal calving interval is fundamental to increase the return of dairy farms. Therefore, minimizing the calving to conception interval may represent economic advantages for dairy producers (Hay et al., 2019). In our group of cows, no differences were found for the calving to conception interval for IgG category or disease in the pre-weaned life. Consequent with our results, a study following calves for 52 months did not find differences in the interval calving to conception (138 -130 days after calving) of cows fed maternal or plasma-derived colostrum after birth (Pithua et al., 2010).

Even though our results were not significant, a numerical difference was observed in the days from calving to conception by IgG category. Cows with Excellent passive immune transfer had a shorter interval when compared to the Poor category cows. The difference between these 2 groups was 62 days; consequently, cows in the Excellent category had a second calving 61 days earlier than cows in the Poor category. This might represent an economic benefit for producers, despite the lack of statistical significance. In addition, cows with an Excellent pre-weaned ADG had a 60-day shorter calving to conception interval, and their second calving was 70 days earlier than cows with Poor ADG.

The presence of disease events in the pre-weaned life did not affect the calving to conception interval or age at second calving. Although, cows in this trial had the second calving between 36 and 39 months of life, the Excellent category for IgG and pre-weaned ADG was the closest to the recommendation of 1 calving per year.

Days in milk in the first lactation

In this group of cows, the mean DIM was over 305 days independent of the early life predictor, and no significant differences were observed. However, cows in the Excellent category for IgG and pre-weaned ADG had on average a DIM closer to 305 days (325 and 311, respectively). Despite this, and as mentioned in the results section, an important percentage of cows had long lactations due to reproductive problems or abortions. Therefore, the mean values for DIM should be taken with caution. To our knowledge, there are no studies assessing the influence of pre-weaned characteristics on DIM in the first lactation.

Milk yield in the first lactation

In our study, no differences were found for total, adjusted, and daily milk yield by IgG category for cows that reach the first lactation or for all the enrolled cows. Concerning the results for total milk yield, it is critical to consider that 51% of the cows had lactations over 305 days. When observing adjusted milk yield to 305 days, there is a significant numerical difference in the milk produced in the first lactation when including only cows that reached the first lactation and when including all the enrolled cows to account for milk not produced. A significant difference in adjusted milk yield was found in a study that followed cows for 52 months. The cows fed plasma derived colostrum had a higher risk of failure in passive transfer than cows fed maternal colostrum, and they produced 429 kg less of milk in the first lactation (Pithua et al., 2010). Faber et al. (2005) found a difference of 2,387 kg of adjusted milk yield favoring cows fed 4L of colostrum rather than 2L of colostrum after birth. However, passive immune transfer was not measured in the enrolled animals; therefore, it might not be related to the transfer of passive immunity.

In relation to pre-weaned ADG, the current study found numerical differences that favored the Excellent ADG category for the adjusted milk yield and daily milk yield. Comparable to our results, numerical differences of 291 and 765 kg of adjusted milk were reported in studies comparing conventional vs. intensified pre-weaned feeding programs in the adjusted milk yield in the first lactation (Davis Rincker et al., 2011; Korst et al., 2017). In addition, for every kg increase of pre-weaned ADG, cows significantly produced 850 and 1,550 kg more adjusted milk in the first lactation (Soberon et al., 2012; Soberon and Van Amburgh, 2013). Furthermore, calves growing at 0.82 kg/d in the pre-breeding, which is in accordance with the Excellent category, produce significantly 1,120 kg more of adjusted milk than heifers growing to 0.55 kg/d (Hayes et al., 2021). One potential explanation for an increased milk yield is that a higher plane of nutrition in the pre-weaned period can positively affect the parenchymal development of the mammary gland (Brown and et. 2005, Gelsinger et al. 2016). Nevertheless, a meta-analysis found that other management practices were more important in determining milk yield in the first lactation than pre-weaned ADG. However, it was indicated that keeping ADG above 0.5 kg/d can improve the performance of cows in the first lactation (Gelsinger et al., 2016).

When the pre-weaned disease event was used as the predictor for total, adjusted, and daily milk yield in cows that reached the first lactation, no differences were found. In contrast, when all enrolled cows were included in the analysis, the milk yield was statistically significant. A recent study evaluated the effect of pre-weaned diarrhea and BRD in the performance of cows at first calving. The authors found that cows that experienced diarrhea had a 325 kg reduction in the adjusted milk yield, and no difference was found for BRD (Abuelo et al., 2021a). Additionally, Heinrichs and Heinrichs (2011) found a negative effect in the number of days with diarrhea or coughing in the first 4 months of life on the adjusted milk yield in the first lactation. Likewise, a study found that cows that experienced mild diarrhea during the first 3 months of life had a reduced adjusted milk yield in the first lactation than cows without diarrhea (Svensson and Hultgren, 2008). Contrarily, no detrimental effect of calf morbidity (dullness, respiratory disease, and diarrhea) was observed in the adjusted milk yield in the first lactation (Warnick et al., 1995; Rossini, 2004). Although, a decrease in the protein content (0.05kg/d) of the milk was found when cows experienced respiratory disease (Rossini, 2004). The long-term impact of pre-weaned health on production is not clear, although evidence suggests an effect.

Leaving the herd

Less is known about the effects of pre-weaned managements in the survival of cows through a lifetime. The culling rate for the cows during the first lactation (26%) was higher than published prospective studies, where an 8.4% and 19% of culling has been described (Bach, 2011; Brickell and Wathes, 2011).

For the complete observation period, a trend was found for the odds of a cow leaving the herds by IgG category. Cows in the Poor category were 1.45 times more likely to leave the herd in comparison to the Excellent category cows. Pithua et al. (2010) did not find differences in the hazard of death or culling from birth to 54 months of life in cows fed plasma-derived colostrum and maternal colostrum after birth; even though the first group mentioned had a higher likelihood of failure in passive transfer. In our knowledge, there are no studies assessing passive immunity and its effect on the longevity of the cows in the herd.

Pre-weaned ADG was not associated with the likelihood or the hazard of cows to leave the herd as heifer, in the first lactation, or the complete period observed from 2014 to 2019. A study following calves to the second lactation found that cows with pre-weaned ADG of 0.8 kg/d were more likely to stay in the herd up to the second lactation than cows with pre-weaned ADG lesser than 0.7kg/d (Bach, 2011).

Heifers that presented at least one health event in the pre-weaned stage were 3.3 times more likely to leave the herd before the first lactation than calves that did not have a health event. Supporting our results, calves treated for pneumonia in the first 3 months of life were 2.5 times more likely to die after 90 days of life than calves not presenting a disease event. Likewise, calves that were treated for diarrhea were 2.5 times more likely to be sold for dairy purposes than other calves (Waltner -Toews et al., 1986). Additionally, it was described that heifers with no disease events in the pre-weaned life had 8% more probability of staying in the herd at 730 days of life than calves with 2 or more disease events (Rossini, 2004).

For the first lactation and the complete period observed, no differences were found in the odds or hazard of a cow to leave the herd by disease presentation in the pre-weaned life. The survival curves joined around day 1,400 of life. However, it was observed a clear separation in the probability to leave the herd by pre-weaned health status from birth up to day 1,400. Similarly, Warnick and White (1995) did not find an association between producer-reported signs of disease in the pre-weaned life and the survival of the heifers after the first calving. Additionally, a prospective study analyzing calf factors associated with survival of cows did not find differences in the age at culling of cows by the number of days ill or days in treatment in the pre-weaned life

(Heinrichs and Heinrichs, 2011). Moreover, diarrhea in the pre-weaned period was not associated to the likelihood of finishing the first lactation (Bach, 2011)

Study limitations

As the analysis was retrospective in nature, data was not recorded with the aim of completing this study; therefore, some animals had missing or incomplete records. In addition, differences in record-keeping were seen across farms. As mentioned throughout the discussion, we observed numerical differences that favored cows with Excellent passive immunity, Excellent ADG, and no disease in the pre-weaned life. No statistically significant differences were found even when our estimates agreed with published data. Potentially, this study lacks the power to demonstrate some of the associations, especially when comparing the sample size to published studies. The current study was performed as an exploratory study with a convenience sample size.

Conclusion

This study analyzed the effect of passive immunity, pre-weaned health, and pre-weaned ADG on productive, reproductive parameters, and survival past the first lactation of Holstein cows. The results indicated an association between pre-weaned ADG and the calving to conception interval during the first lactation and, consequently, the age at the second calving. In addition, we observed an increased likelihood to leave the herd before the first calving when heifers had at least one health event in the pre-weaned life. It is encouraged to develop more research focused in this area, with a larger number of animals in multiple operations.

Tables and Figures

Table 2.1: Descriptive statistics for the distribution of cows by variables used in the analyses

Variable	Calves, n(%)	Cumulative Frequency	Cumulative Percent
Farm			
1	50 (26.0)	50	26
2	62 (32.3)	112	58.3
3	46 (24.0)	158	82.3
4	34 (17.7)	192	100
Year			
2014	107 (55.7)	107	55.7
2015	85 (44.3)	192	100
Season			
Summer	69 (35.9)	69	35.9
Fall	29 (15.1)	98	51
Winter	42 (21.9)	140	72.9
Spring	52 (27.1)	192	100
IgG category			
Excellent (≥ 25g/L)	78 (40.6)	78	40.6
Good (18.0-24.9 g/L)	57 (29.7)	135	70.3
Fair (10.0-17.9 g/L)	36 (18.8)	171	89.1
Poor (<10.0 g/L)	21 (10.9)	192	100
ADG category			
Excellent (> 0.82 kg/d)	71 (42.8)	71	42.8
Fair (0.64 to 0.82 kg/d)	51 (30.7)	122	73.5
Poor (< 0.64 kg/d)	44 (26.5)	166	100
Disease pre-weaned			
No	129 (67.2)	129	67.2
Yes	63 (32.8)	192	100

Table 2.2: LSM for reproductive parameters by IgG category, disease presentation in the pre-weaned life, and pre-weaned ADG category. Age at conception (n=146), age at first calving (n=146), days in milk (DIM) in the first lactation (n=146), calving to conception interval (n=108), and age at second calving (n=108). Different letters within a column and predictor variable indicate significant differences.

	Age at conception (d)			Age 1st calving (d)			DIM 1stlactation			Calving to conception interval (d)			Age 2nd calving (d)		
Predictor	LSM	SE	*P*	LSM	SE	*P*	LSM	SE	*P*	LSM	SE	*P*	LSM	SE	*P*
IgG			0.05			0.05			0.98			0.21			0.17
Excellent	446.3	25.89		724.3	25.89		325.5	15.64		115.2	17.12		1122.8	36.86	
Good	455.9	26.17		733.9	26.17		324.8	20.12		147.2	21.6		1163.1	39.46	
Fair	468.9	26.56		746.9	26.56		325.9	25.4		129.1	25.9		1151.9	42.17	
Poor	439.2	27.85		717.2	27.84		338.3	36.85		177.6	33.01		1183.7	47.76	
Disease			0.91			0.91			0.38			0.56			0.74
No	452.9	26.61		730.9	26.61		320.6	13.13		132.8	15.22		1143.4	35.59	
Yes	452.1	27.10		730.1	27.10		341.3	21.04		120.6	21.37		1136.1	38.94	
ADG			0.59			0.59			0.11			0.03			0.02
Excellent	448.7	26.70		726.7	26.70		311.9	17.00		99.7[a]	16.59		1108.7[a]	34.37	
Fair	457.0	26.85		735.0	26.86		310.6	20.08		148.5[a,b]	19.09		1160.6[a,b]	35.91	
Poor	455.4	27.03		733.4	27.03		364.2	21.26		159.6[b]	20.38		1178.7[b]	36.84	

Table 2.3: LSM for milk yield (kg) in the first lactation by IgG category, disease presentation in the pre-weaned life, and pre-weaned ADG category, including only cows that reached the first lactation (n=146).

	Milk yield 1st lactation (kg)			Adjusted milk yield 305d (kg)			Daily milk yield (kg/d)		
Predictor	LSM	SE	*P*	LSM	SE	*P*	LSM	SE	*P*
IgG			0.95			0.65			0.65
Excellent	10,324	1,383		9,439	1,001		30.9	3.28	
Good	9,968	1,449		9,103	1,024		29.8	3.36	
Fair	10,321	1,543		8,855	1,057		29.0	3.47	
Poor	10,717	1,817		8,986	1,161		29.5	3.81	
Disease			0.39			0.49			0.49
No	10,065	1,363		9,139	997		30.0	3.27	
Yes	10,772	1,478		9,419	1,038		30.9	3.40	
ADG			0.64			0.12			0.12
Excellent	9,944	1,341		9,515	1,055		31.2	3.46	
Fair	10,171	1,379		9,449	1,066		31.0	3.50	
Poor	10,881	1,422		8,552	1,080		28.0	3.54	

Table 2.4: LSM for milk yield (kg) in the first lactation by IgG category, disease presentation in the pre-weaned life, and pre-weaned ADG category including all the cows enrolled in the study (n=192).

	Milk yield 1st lactation all cows (kg)			Adjusted milk yield all cows (kg)			Daily milk yield all cows (kg/d)		
Predictor	LSM	SE	*P*	LSM	SE	*P*	LSM	SE	*P*
IgG			0.64			0.23			0.24
Excellent	8,569	1,108		7,854	833		25.8	2.78	
Good	7,744	1,190		7,029	909		23.0	2.98	
Fair	7,101	1,331		6,107	1,017		20.0	3.33	
Poor	7,524	1,715		6,322	1,310		20.7	4.30	
Disease			0.04			0.007			0.006
No	8,576	999		7,744	424		25.6	2.44	
Yes	6,684	1,140		5,798	597		19.1	2.80	
ADG			0.93			0.56			0.29
Excellent	8,440	1,167		8,044	881		26.4	2.89	
Fair	8,848	1,243		8,259	936		27.1	3.07	
Poor	8,735	1,167		6,924	968		22.7	3.18	

Table 2.5: Odds ratio for leaving the herd before first calving (n=192), during the first lactation (n=146) and for the complete period observed (n=192; June 2014 to August 2019) by serum IgG Category, pre-weaned health status, and pre-weaned ADG.

	Left as heifer			Left 1st lactation			Left complete period		
Predictor	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
IgG category			0.33			0.48			0.05
Excellent vs Poor	0.48	0.15-1.56		0.99	0.21-4.68		0.69	0.21-2.23	
Good vs Poor	0.89	0.28-2.82		1.69	0.36-8.10		1.83	0.51-6.56	
Fair vs Poor	1.05	0.31-3.60		2.1	0.40-11.01		2.03	0.52-7.99	
Disease			0.001			0.82			0.99
disease vs no disease	3.30	1.63-6.69		1.11	0.46-2.67		0.99	0.49-2.03	
ADG category			0.77			0.79			0.49
Excellent vs Poor	0.76	0.28-2.05		1.04	0.37-2.94		1.53	0.64-3.69	
Fair vs Poor	0.68	0.24-2.01		1.38	0.47-4.04		1.66	0.66-4.20	

Table 2.6: Hazard ratio for the time to leaving the herd before first calving, during the first lactation, and for the complete period by serum IgG Category, pre-weaned health status, and pre-weaned ADG.

	Left as heifer			Left 1st lactation			Left complete period		
Predictor	HR	95%CI	*P*	HR	95%CI	*P*	HR	95%CI	*P*
IgG category			0.34			0.51			0.04
Excellent vs Poor	0.49	0.18-1.34		0.90	0.29-4.27		0.67	0.33-0.88	
Good vs Poor	0.81	0.31-2.14		1.46	0.38-5.48		1.12	0.60-2.13	
Fair vs Poor	0.95	0.34-2.62		1.43	0.39-6.75		1.23	0.62-2.44	
Disease			0.0005			0.95			0.15
disease vs no disease	2.83	1.57-5.10		1.02	0.49-2.12		1.31	0.90-1.88	
ADG category			0.79			0.68			0.69
Excellent vs Poor	0.79	0.32-1.89		1.01	0.41-2.43		1.10	0.65-1.83	
Fair vs Poor	0.74	0.28-1.92		1.35	0.55-3.31		1.24	0.74-2.12	

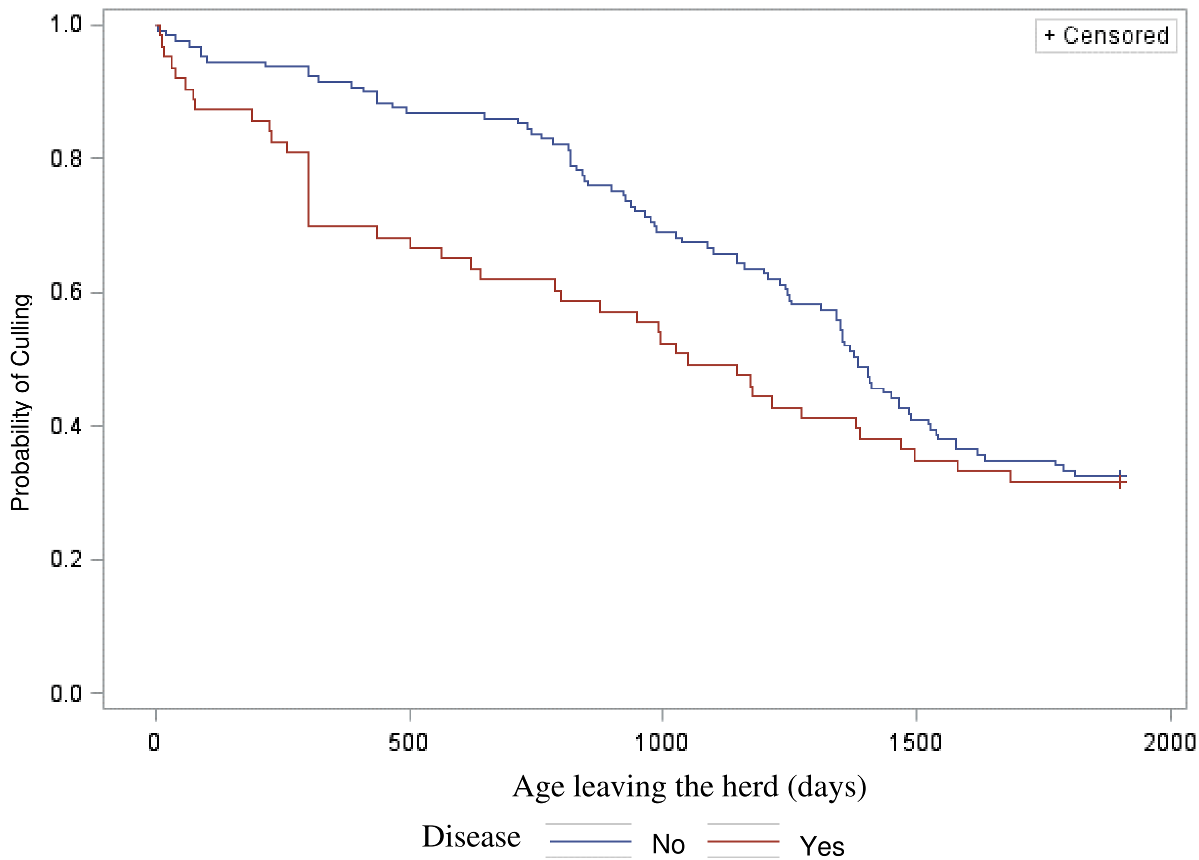

Figure 2.1: Survival curve for the time to culling from birth to 1,900 days of life by health status in the pre-weaned life (n=192 cows). Blue line=no health events in the pre-weaned life; Red line= at least 1 health event before weaning ($P_{\text{leaving before first calving}}$= 0.001; $P_{\text{complete period}}$=0.99). Data included in the analysis were from June 2014 to August 2019.

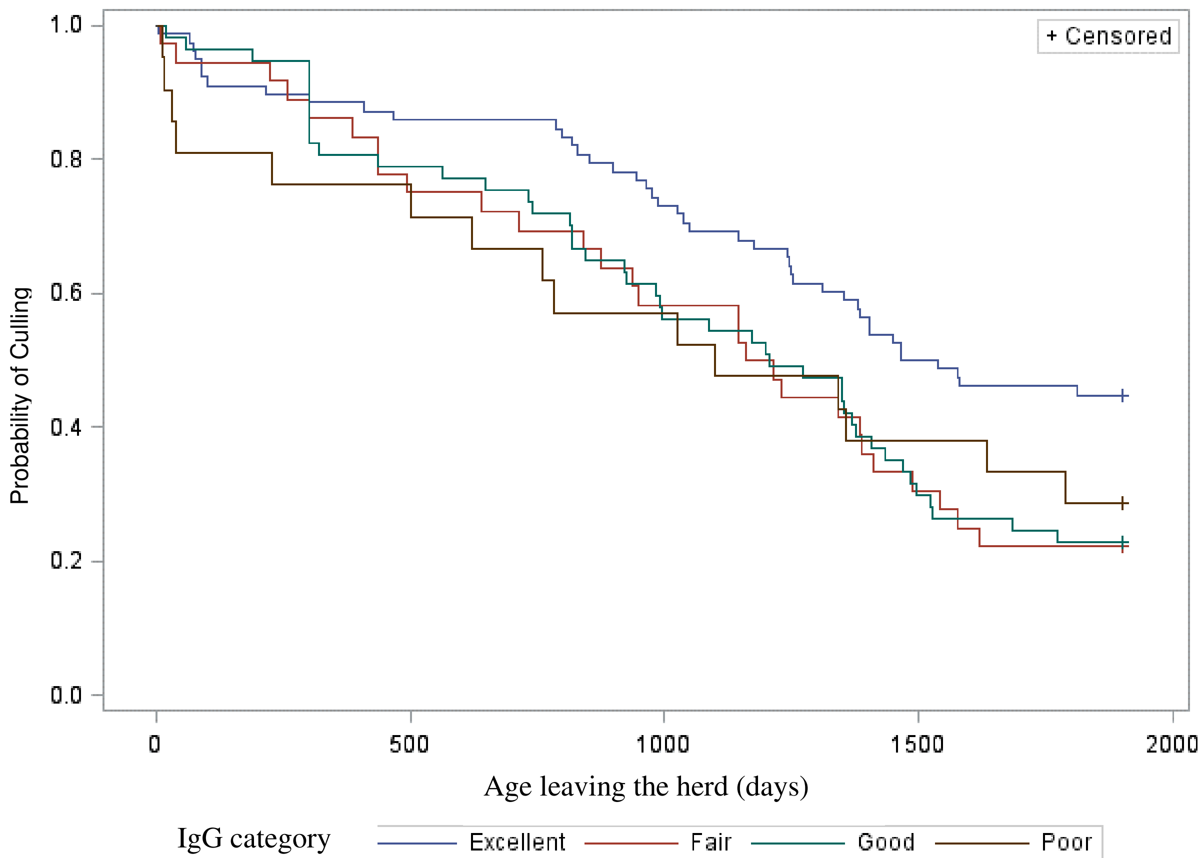

Figure 2.2: Survival curve for the time to culling from birth to 1,900 days of life by serum IgG category (n=192 cows). Blue line= Excellent (≥ 25g/L); Green line= Good (18.0-24.9 g/L); Red line= Fair (10.0-17.9 g/L); and Brown line= Poor (<10.0 g/L; P=0.05). Data included in the analysis were from June 2014 to August 2019.

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CHAPTER 3: EVALUATION OF A PROBIOTIC PROGRAM ON HEALTH AND PERFORMANCE OF ORGANIC CERTIFIED HOLSTEIN HEIFER CALVES

Introduction

Diarrhea is the most important cause of morbidity and mortality in young calves (McGuirk, 2008a; USDA, 2011; Urie et al., 2018). In the US, between 18 to 21% of pre-weaned calves suffer diarrhea, and 75.9% of the cases are treated with antimicrobials (Urie et al., 2019; USDA, 2018). Diarrhea not only causes detrimental effects on the health and performance of calves, but it also has an economic impact on the producer due to animal losses and treatments (McGuirk, 2008).

There is evidence suggesting that healthy animals have a balanced gut microbiota, which allows them to grow adequately (Signorini et al., 2012; Uyeno et al., 2015). The bacterial colonization of the gastrointestinal tract of calves starts at calving. However, management practices and intense productive systems, expose young dairy calves to stressful conditions that might lead to microbiota disruption (Indart et al., 2012; Cangiano et al., 2020). Additionally, pre-weaned calves are highly susceptible to digestive diseases in the first month of life (Hubert and Moisá, 2016).

As consumer concern for the use of antibiotics in food animals increases, the identification of alternative preventive and therapeutic strategies to enhance cattle health has become more relevant (Xiong et al., 2018). Probiotics offer a novel approach for disease management that could reduce the use of antimicrobials (Indart et al., 2012). The FAO/WHO defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al., 2014). Currently, two main types of probiotics are used in dairy calves; yeast culture probiotics and bacterial based probiotics; both can be administered in milk or calf starter.

Three major concepts had been proposed as mechanisms of action for probiotics. First, the interaction with host cells by enhancing the epithelial barrier; second, the inhibition of pathogen adhesion and growth; and third, the modulation of the immune response of the host (Khare et al., 2018; Cangiano et al., 2020, Alawneh et al., 2020). Therefore, supplementing newborn and pre-weaned calves with bacterial-based probiotics might assist them in keeping a balanced gut microbiota and enhancing their health from birth to weaning. Supporting this concept, previous reports indicate reductions in diarrhea presentation, and severity, along with an improved performance (Timmermann et al., 2005; Frizzo et al., 2010; Cantor et al., 2019). Thus, we hypothesized that the administration of a two steps probiotic protocol (colostrum and milk) would reduce the presentation of neonatal diarrhea, improving overall health and performance during the pre-weaned life.

The objective of this study was to evaluate the effect of a probiotic program on the digestive health of calves during the pre-weaning period. Specific outcomes were age at the first diarrhea event, time to recovery, the total number of diarrhea events, and average daily gain from birth to weaning.

Material and Methods

This randomized clinical trial was conducted in an organic-certified dairy calf rearing facility in Northern Colorado from July 21st to October 16th, 2020. The study was performed in accordance with the guidelines set by Colorado State University Institutional Animal Care and Use Committee (protocol ID: 1324).

Study population and farm managements

Calves born in the maternity barn were routinely separated from the dam after birth. Newborn calves were housed in a pen with protection from environmental conditions and clean straw bedding. Navels were disinfected after birth with 7% Iodine.

The first colostrum feeding was provided within 1 hour after birth. A successful first feeding was considered when calves consumed a minimum of 4L of pasteurized high-quality colostrum (≥52 mg/ml immunoglobulins) in the first 4 hours of life. Two extra liters of colostrum were offered at 12 and 24 hours of life. All calves were bottle-fed, and colostrum quality was assessed with a colostrometer (Biogenics, Florence, OR).

Farm personnel selected heifer calves before transferring to the rearing facility within the first day of life. The selection criteria included 2 main factors: Calves must had been born from eutocic births and voluntarily consumed 4L of high-quality colostrum in the first 4 hours of life. The selected calves were ear-tagged by date and hour of birth.

Calves moved to the rearing facility were housed in pairs following sequential ID numbers. The housing, or calf unit, included 2 individual polyethylene calf hutches (Agri-Plastics, Stoney Creek, ON, Canada) enclosed by a wire fence in a total area of $4.5m^2$/pair. Calf units were set in rows of 50, separated by approximately 1 meter from each other. Two bottle holders were placed in opposite corners in the front of the enclosed pen. A wire wall of 86 cm was placed in each bottle holder area; it was set as a physical barrier to avoid displacements at feeding time. Two buckets for water and two buckets for organic certified calf starter (16% Organic Calf Starter, Feedex Companies, LLC. South Hutchinsin, KS) were positioned in the front of the enclosing gate. Water was offered since arrival, and calf starter was offered since day 4 of life.

The first feeding at the rearing facility consisted of 2L of high-quality colostrum. Three milk feedings were provided per day at 6:30 AM, 1:00 PM, and 7:30 PM. Whole milk was pasteurized and offered in nursing bottles of 1.8L and 2.8 L. The daily milk allowance from day 1 to day 7 of life was 5.6 L, from day 8 to 48 of life was 10.4 L, from day 49 to 55 of life was 5.6 L, from day 56 to 63 of life was 2.8 L of milk, finally, from day 64 to 69 only water was provided. Step down weaning started at day 49 and finished at day 69 of life, when the calf starter intake was 4 to 5 lb.

The vaccination protocol included intranasal Inforce 3 (IBR, PI3, BRSV; Zoetis, Florham Park, NJ) at 14 days of life, subcutaneous Bovishield Gold FP5 L5 (IBR, BVD [Types 1 and 2], PI3, BRSV, *Leptospira Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona*; Zoetis, Florham Park, NJ), and subcutaneous Occuguard-MB1 (*Moraxella bovis;* Boehringer Ingelheim, Duluth, GA). Subcutaneous vaccines were administered between 46 to 52 days of life. Hot iron dehorning was performed by trained personnel between 15 and 21 days of life. The protocol included restriction of movement, local anesthesia, and analgesia.

Daily calf health assessments were performed by trained farm personnel, and treatments were provided according to the farm SOP. Calves needing treatments not allowed under organic regulations were sold. Euthanasia, when necessary, was performed by certified workers under the supervision of the farm veterinarian.

Treatment description

The experimental treatment consisted of two formulations of a bacterial-based probiotic (Establish Calf-O and Young-O; Perdue AgriBusiness Llc., Salisbury, MD; Table 3.1). The administration of the probiotics was in accordance with the manufacturer's recommendations. Briefly, Formulation 1 [PBF1] was mixed in pasteurized colostrum in a dose of 2gr per feeding.

This formulation contained 5 billion CFU/dose of *Enterococcus faecium, Lactobalillus casei KE01, Lactobacillus acidophilus, Bifidobacterium bifidum, and Bifidobacterium longum*. Additionally, Formulation 2 [PBF2] was mixed in pasteurized milk 3 times per week in a dose of 1gr per day. This formulation contained 5 billion CFU/dose of *Enterococcus faecium, Lactobacillus casei KE01 supernatant, Bacillus sutilis, Bacillus licheniformis* (Table 3.1).

Experimental design and data collection

A total of 232 calves were included in this study (control [CTR]= 116, probiotic [PB]= 116). Heifer calves were enrolled at birth and followed until weaning.

Calves in the PB group received 2gr of PBF1 in each colostrum feeding (2 to 4 depending on the hour of birth) and 1 gr of PBF2 in milk 3 times per week until weaning. CTR calves did not receive any product on colostrum or milk.

Farm personnel was responsible for providing PBF1 to newborn calves. As the maternity pen had 3 work shifts, one of the researchers provided training to all workers. Daily visits to the maternity were scheduled, and birth data were collected. At the rearing facility, PBF2 was provided in the morning milk feeding (6:30 AM) on Monday, Wednesday, and Friday. Two researchers prepared the treatment bottles at the pasteurization time. Due to a large number of bottles, farm personnel distributed CTR and PB bottles under the supervision of the researchers. To avoid misplacing of treatment and control bottles, an elastic band was attached to each treatment bottle.

Serum total protein (STP) was measured from all calves at 3±1 days of life. A minimum of 5 ml of blood was collected via jugular venipuncture by using a vacutainer and a tube without anticoagulant (Becton Dickinson Vacutainer, Franklin Lakes, NJ). Blood samples were allowed to clot and centrifuged for 15 minutes at 2,200 rpm. A digital refractometer (PA200-013 Palm Abbe, Misco Refractometer, Solon, OH) was used to quantify the STP concentration.

A health assessment was performed on all enrolled calves 3 times per week, on the same days that PBF2 was provided. The assessment included the Wisconsin calf health chart (McGuirk, 2008b), dehydration score (Wattiaux, 2005; Smith, 2009), attitude score (Perino and Apley, 1995), and percentage of milk consumed. All scores were on a 4-point scale (Table 3.2). For analysis, health scores were dichotomized. Fecal scores 0 and 1 were considered normal, and fecal scores 2 and 3 were considered diarrheic. In addition, score 3 was also considered severe diarrhea. Similarly, all health categories were considered normal at score 0 or 1, and abnormal at score 2 and 3. Recovery from a diarrhea event was established when 2 consecutive fecal scores were 0 or 1 and no other clinical signs of disease were observed. The first day with normal scores was considered as the recovery date.

For estimation of weight gain and ADG, one researcher measured the body weight from all the enrolled calves with the Holstein calf weight (The Coburn Company Inc., Whitewater, WI) at birth, 30±2 days, and 65±2 days of life.

Dates and causes for calves leaving the facility were recorded. In this study, culling was considered as any calf that left the rearing facility due to death, sale to another facility (disease or antibiotic administration), and euthanasia.

A subsample of calves (CTR=25, PB=25) was randomly selected for the collection of additional blood samples for serum haptoglobin (Hp) quantification at 7, 14, 21, 30, 40, 50, and 65 days of life (±2d). Samples were collected and processed as previously described for STP; one aliquot of serum was frozen at -80°C until the end of the study period. A direct ELISA kit was used to quantify the serum concentration of Hp (Bovine Haptoglobin ELISA Kit, Immunology Consultants Laboratory, Portland, OR). From the same calves, a fecal sample was collected at 7, 14, and 21 days of life for a pathogen screen (Clostridium, Cryptosporidium, *Salmonella*, Coronavirus, Rotavirus, and BVDV). Samples were collected via rectal stimulation into sterile tubes of 50ml. Fecal samples were submitted fresh to CSU Veterinary Diagnostic Laboratory (Fort Collins, Colorado) and results were provided as detected or not detected.

Lung ultrasonography (US) was performed weekly on a subsample of 20 calves (CTR=10, PB=10) to assess respiratory health. The assessment of the 2 lungs was performed systematically as described by Ollivet and Buczinski (2016). Briefly, calves were momentarily restrained, and 70% isopropyl alcohol was sprayed on the hair as a transducing agent. A portable ultrasound (Easi-Scan, IMV imaging, Rochester, MN) was used to evaluate lungs from the caudal to the cranial thorax, moving the probe from dorsal to ventral within each intercostal space. Healthy was defined as lung consolidation $< 1cm^2$ with no clinical signs; clinical BRD was defined as lung consolidation $\geq 1cm^2$ with clinical signs, and subclinical BRD was defined as lung consolidation $\geq 1cm^2$ with no clinical signs (Cramer et al., 2018).

As this trial was performed in summer, temperature and humidity were measured during the complete study period. Two HOBO Pro v2 loggers (Onset Computer Corporation., Bourne, MA) were placed in the rearing facility to obtain ambient temperature and humidity. Additionally, 2 HOBO UX100-011 temp/RH 2.5% loggers (Onset Computer Corporation., Bourne, MA) were installed inside 2 empty hutches. Temperature and humidity were set to be recorded every 30 minutes. The temperature-humidity index (THI) was calculated using the equation $THI = (1.8 \times T + 32) - ((0.55 - 0.0055 \times RH) \times (1.8 \times T - 26))$, where T = temperature (°C) and RH = relative humidity (Kendall et al., 2008; Vickers et al., 2010; Manriquez et al., 2018).

For consistency, health assessments, lung US, and body weight were collected by one researcher during the complete study period. All management practices were similar for the treatment groups, except for the administration of PBF1 and PBF2 in colostrum and milk of the PB calves.

Statistical analysis

All statistical analyses were performed on SAS version 9.4 (SAS Institute Inc., Cary, NC). Data was analyzed using the calf as experimental unit, calves were clustered by pair number. A significant level was established at $P<0.05$.

Exploratory analyses were performed with PROC MEANS and PROC FREQ. In the case of continuous variables, normality was assessed with PROC UNIVARIATE with Shapiro-Wilk statistics. Homogeneity of variance was assessed with PROC GLM.

Data corresponding to THI was organized in Microsoft Excel for Microsoft 365 (Microsoft Corporation, Redmond, WA). Ambient and hutch THI were graphed by hour for the complete study period.

Time to event analysis (PROC LIFETEST) was performed to calculate the median time to a first diarrhea episode, the time to recovery from a first diarrhea episode, and the time to culling by treatment group. The proportional hazard ratio (PROC PHREG) was performed to calculate the hazards of calves of having a first diarrhea episode and for recovering from the first diarrhea episode by treatment group while clustering the calf by pair number.

Generalized linear mixed model (PROC GLIMMIX) with logit link and binary distribution was used to analyze the odds of presenting other clinical signs of disease (besides diarrhea) in the first diarrhea event, and for the odds of culling by treatment group while considering calves by pair number as a cluster for analysis.

Poisson regression (PROC GENMOD) was used to identify differences in the rate of diarrhea events in the study period, and for the rate of calves with severe diarrhea by treatment group. The model was clustered by calf pair and corrected by total number of health assessments per calf.

For continuous variables, such as STP concentration and birth weight, a T-Test was performed using PROC TTEST to detect differences by treatment group. Consequently, a linear model (PROC GLM) was built to estimate the difference in the LSM of ADG by treatment group adjusted by birth weight.

The frequency (PROC FREQ) for fecal pathogen presentation and for subclinical BRD was calculated for each sampling point. A repeated measures analysis for a binary response (PROC GENMOD) was performed to calculate the odds of the calves to be positive to Clostridium by treatment group and sample number; the model included the interaction term for treatment group and sample number.

Repeated measures analysis (PROC MIXED) was used to identify differences in the LSM for serum haptoglobin and for milk intake by treatment group and time; the model included the interaction between treatment group and time. Lastly, the week of birth was considered as a potential confounder in the analyses.

Results

A total of 232 calves (CTR=116; PB=116) were enrolled in a period of 4 weeks (week 1= 53 calves; week 2 =47 calves; week 3= 80 calves; week 4= 44 calves). Calves were followed from birth to culling or weaning. No differences were found in the outcomes when adjusting by the week of birth.

Weather conditions

The study was conducted during Summer and Fall 2020 in Colorado. The ambient THI ranged from 26 to 81 units, while THI inside the hutch ranged from 23 to 86 units. A detailed description of daily THI is presented in the Appendix section (A3.1). Ambient and hutch THI by hour for the complete study period is shown in Figure 3.1.

During day hours (8 AM to 7 PM) calves were exposed to THI over 70 units for the first 7 weeks in study (July 21st to September 8th, 2020). Furthermore, THI inside the hutch was on average 2 units greater than ambient THI. Calves were exposed to THI over 80 units inside the hutch during the day hours. On the 7th week in study (September 8th), a drastic drop in THI was experienced due to a winter storm. In the days following the storm, THI increased over 70 units in day hours, although calves were exposed for fewer hours to high THI. Additionally, the average THI difference between day and night hours was 10 and 15 units for ambient and hutch, respectively.

Baseline measurements

Birth weight (mean±SE) was greater in CTR (39.18±0.17 kg) than in PB (38.68±0.17 kg); P= 0.04; Table 3.3). Serum TP concentrations did not differ by treatment group (CTR= 6.75±0.06 g/dL; PB= 6.60±0.06 g/dL; P=0.06; Table 3.3). Based on the most recent STP categorization by Lombard et al. (2020), 186 (80.2%) calves were in the Excellent category [CTR: 93 (40%); PB: 93 (40%)]; 26 (11.2%) calves in the Good category [CTR= 12(5.2%); PB= 14(6.0%)]; 18(7.8%) in the Fair category [CTR= 11(4.8%); PB= 7(3.0%)]; and 2(0.9%) in the Poor category [CTR= 0(0%); PB= 2(0.9%)].

Presentation of diarrhea and recovery time

The overall incidence of diarrhea was 96.6% (CTR=99.1%; PB=94.9%), with 224 calves (CTR=115; PB =109) having at least 1 diarrhea event in the study period. The median time [SE] to the first diarrhea event did not differ by treatment group (CTR= 11d [10-12d]; PB= 11d [10-12d]; P=0.97; Figure 3.2; Table 3.4). The Cox proportional analysis did not find a difference in the hazard (95%CI) of presenting a first diarrhea event by treatment group (HR [CTR vs. PB]= 1.05 [0.82-1.36]; P=0.69; Table 3.4).

From all calves presenting a diarrhea event, 67 of them had other signs of clinical disease (CTR= 38; PB= 29), mainly dehydration or abnormal attitude. There was no significant association between treatment and dehydration and/or an abnormal attitude in the first diarrhea event (OR [CTR vs. PB] = 1.40 (0.69-2.92); P=0.33; Table 3.4).

In total, 202 calves recovered from the first diarrhea episode (CTR= 104; TRT=98). No difference was observed in the median time to recovery [SE] by treatment group (CTR=7d [5-7d]; PB=7d [5-9d]; P=0.41; Figure 3.3; Table 3.4). Likewise, there was not a difference in the likelihood (95% CI) of recovering from a first diarrhea event, when comparing CTR vs. PR calves clustered by pair (HR= 1.07 (0.81-1.42); P=0.69; Table 3.4).

The number of diarrhea events by calf ranged between 0 to 4 in both groups. No difference was observed in the rate (95%CI) of new diarrhea events by treatment group [rate=1.01 (0.89-1.15); P=0.86; Table 3.4].

Lastly, the total number of clinical examinations with severe diarrhea (score 3) ranged from 0 to 10 for CTR calves and 0 to 9 for PB calves. There was no significant difference in the rate (95% CI) of severe diarrhea exams comparing CTR vs. PB calves clustered by pair [rate=0.93 (0.72-1.23); P=0.63; Table 3.4].

Performance

Body weight was measured at 30±3 days of life (CTR=108; PB= 103 calves) and 64±3 days of life (CTR= 93; PB=96 calves). No difference was found in ADG by treatment group while adjusting by birth weight at 30 days (CTR= 437.67 ± 22.95 gr/d; PB= 445.37 ± 23.59 gr/d; P=0.71; Table 3.3) or at weaning (CTR= 562.47 ± 13.92 gr/d; PB=570.79 ± 13.53 gr/d; P=0.67; Table 3.3).

Culling

During the complete study period 41 calves (CRT=21 [18.10%]; PB=20 [17.24%]) left the facility due to death, (CTR=10 [8.62%]; PB=13 [11.20%]), sold to another facility (CTR=10 [8.62%]; PB=4 [3.45%]), and euthanasia (CTR=1 [0.86%]; PB=3 [2.58%]). The 3 main causes for culling were respiratory (CTR=14 [12.06%], PB=10 [8.62%]), digestive (CTR=6 [5.17%], PB=9 [7.75%]), bloat (CTR=1 [0.86%], PB=1 [0.86%]).

The odds (95%CI) of a calf to leave the facility when comparing CTR versus PB calves was not significant (OR=1.06 (0.45-2.55); P=0.88, Table 3.4). The time to event analysis did not find differences in the mean time (±SE) to leave the facility by treatment group (CTR= 51.1 ±0.98d; PB= 48.4 ±1.11d; P= 0.94; Figure 3.4; Table 3.4)

Overall, 23 calves died in the facility representing 10.6% of the enrolled calves. In total, 16 calves (6.89%) died due to digestive reasons (CTR= 6 [5.17%], PB= 10 [8.62%]); and 7 calves (3.02%) died due to respiratory causes (CTR= 4 [3.44%], PB= 3 [2.58%]). Out of the 14 calves sold to another facility, only 1 CTR calf was sold due to digestive disease, and 13 calves for respiratory disease (CTR= 9 [7.75%]; PB= 4 [3.44%]).

Fecal Screen

A total of 50 calves (CTR=25, PB= 25) were sampled at 3 points (day 7, 14, and 21 of life) for a complete fecal screen. The most prevalent pathogen was Clostridium. There was a tendency for the odds of calves to be positive to Clostridium after adjusting for repeated measures (OR [CTR vs. PB] = 1.98 (95% CI: 0.96-4.05; P=0.06). Cryptosporidium was the second most prevalent pathogen, with 29 and 33 samples positive in CTR and PB group, respectively (P=0.53). *Salmonella*, Coronavirus, Rotavirus, and BVDV were found in a small number of samples. Table 3.5 provides specific information for each pathogen at each sample point.

Serum Haptoglobin

Fifty calves were sampled at 8 points (3, 7, 14, 21, 31, 41, 51, 65 days of life) for the quantification of serum haptoglobin. The interaction term for treatment group and sample time was statistically significant (P=0.02; Figure 3.5). Sample time 1 (CTR = 187.4±22.52 ug/ml, PB = 119.9±22.5 ug/ml), 2 (CTR = 197.4±22.5 ug/ml, PB = 278.8±22.51 ug/ml) , and 5 (CTR = 202.31±23.44 ug/ml, PB= 153.39±23.07 ug/ml) differed by treatment group.

Lung Ultrasound

Twenty calves were followed with weekly ultrasound from the second week of life to weaning for the detection of subclinical pneumonia. Table 3.6 presents the number of calves with lung consolidation in the absence of clinical signs. In total, 5 CTR and 5 PB calves presented subclinical pneumonia. One calf of each treatment group developed clinical signs of respiratory diseases.

Milk intake

Intake was assessed in the morning feeding on the days the probiotic was provided. An estimation of the consumption was made by calculating the percentage consumed from the amount offered. Overall, calves consumed as average over 95% of the offered milk during the complete study period. The results of the repeated measure analysis detected a difference in the percentage consumed for the interaction term of feeding number and treatment group, favoring PB calves (P=0.0001; Figure 3.6).

Discussion

The study was designed to assess the effect of a bacterial based probiotic strategy on health and performance of pre-weaned Holstein dairy heifer calves.

Weather conditions

This study was performed during Summer and Fall 2020 in Colorado. As shown in the results, these seasons were characterized by THI that surpassed 70 units in most of the days in study. The THI inside the hutch was between 2 and 4 units higher in the day hours than the ambient THI. Due to the setting of the modified housing system, shade was not provided. Additionally, fluctuations between day and night were over 10 units, which might indicate that calves were exposed to important changes in THI during a lapse of 24 hours. Furthermore, a winter storm occurred when calves were 1 to 1.5 months old. As in the first weeks of life, calves are highly susceptible to stress and diseases (Hubert and Moisá, 2016); these weather conditions might have led to significant stress in the study population.

Unlike adult cattle, there is no agreement in a cut-off point to define heat stress in young dairy calves. A study found that calves presented physiological changes, such as increased respiratory rate, rectal and skin temperature, with THI between 65 to 69 units when compared with calves provided heat abatement (Dado-Senn et al., 2020a). Moreover, negative effects on health and wellbeing had been described when calves are exposed to THI over 70 units. Dado-Senn et al. (2020b) found that calves exposed to cooling in the pre-weaned period had a tendency for reduced treatments for fever and infection than heat-stressed calves.

In relation to performance, calves exposed to cooling might have a greater consumption, ADG, and feed efficiency (Hill et al., 2011; Dado-Senn et al., 2020a). Additionally, a national study, including over 2,500 calves, found that calves exposed to THI >70 units had a lower ADG in comparison to calves exposed to THI between 50 to 69 units or THI below 50 units (Shivley et al. 2018). Calves born in summer had a tendency for a lower ADG than calves born in winter months (Place et al., 1998).

Potentially, the calves enrolled in this study might have suffered in-utero heat stress in the last month of gestation. Gestational heat stress may also contribute to impaired immunity, health, and performance in early life and adulthood of dairy cattle (Tao et al., 2013; Monteiro et al., 2016; Dahl et al., 2016; Ling et al., 2017; Ouellet et al., 2020; Dado-Senn et al., 2020c). As gestational heat stress was an unexpected outcome, we do not have data to assess whether this group of animals suffered in-utero heat stress.

All this information is relevant for this study because the outcomes might have been affected by environmental stress due to elevated THI.

Baseline measurements

Levels of passive immunity in the facility were in accordance with the latest recommendation for monitoring passive immunity in the US (Lombard et al., 2020). It has been recommended that at least 40% of calves should be in the excellent category (STP>= 6.2 g/dL) and less than 10% in the poor category (STP < 5.1 g/dL). In our study, 80% of calves reached the Excellent category, and less than 1% were in the Poor category. As no differences were observed in the treatment groups, the enrolled calves started in a similar immune condition. In terms of birth weight, CTR calves were on average 500g heavier than PB calves. A national study reported a greater average birth weight (43.2±0.14 kg) for Holstein heifer calves (Shivley et al., 2018). Therefore, calves in this study were lighter than the national average.

Presentation of diarrhea and recovery time

Diarrhea is the most common disorder affecting pre-weaned calves. The Dairy Heifer Raiser 2011 described that digestive problems affected 25.3% of pre-weaned calves (USDA, 2012). Additionally, a national study that included data from 2,545 calves reported a morbidity of digestive clinical signs of 17.2%, representing 50.9% of all the animals reported sick for the study (Urie et al., 2018). In our study, the incidence of diarrhea was greater than expected (96.6%). Almost all enrolled calves experienced at least 1 diarrhea event (CTR=99.1%; PB=94.9%). Importantly, all enrolled pairs presented at least 1 diarrhea episode. A high incidence of diarrhea in the pre-weaned period is not an uncommon finding in research studies that use probiotics as a prophylactic strategy. As example, the incidence of diarrhea or digestive diseases ranged from 82.0% to 99.7% (Magalhaes et al., 2008; Cantor et al., 2019; Pisoni and Relling, 2020).

It has been described that bacterial-based probiotics might have a positive effect by reducing the presentation of diarrhea. Timmermann et al. (2005) found an 11% reduction in the presentation of severe diarrhea when a multi-strain probiotic was administered to veal calves. Moreover, a meta-analysis described a significant reduction in the pooled estimate for the relative risk of presenting diarrhea in calves receiving bacterial-based probiotics (RR=0.44; 95%CI=0.25-0.76; Signorini et al., 2012). However, the reduction in the presentation of diarrhea was observed only in trials where calves were provided a multi-strain probiotic and fed whole milk. Even though our study tested a multi-strain probiotic, and it was provided in pasteurized colostrum and pasteurized whole milk, no differences were observed in diarrhea incidence in the pre-weaned life.

In our perspective, an important factor in the high incidence of diarrhea was the THI that calves were exposed from birth to the first month of life.

In this study, the probiotic program was not associated with a delay in the onset of diarrhea or a reduction in the hazards of diarrhea. Consistent with our results, the median time for diarrhea presentation has been reported between 10 and 11 days of life (Renaud et al., 2019; Pempek et al., 2019). In addition, the mean age to the onset of diarrhea was described as 6 days of life in a supplementation trial with oregano essential oil (Panagiotis et al., 2017). In general, increased morbidity of diarrhea has been described for the first 2 weeks of life (Foditsch et al. 2015; Urie et al., 2018).

Published trials using bacterial-based probiotics have reported a faster recovery from neonatal diarrhea compared to our study. Renaud et al. (2019) found a mean recovery time for diarrhea of 5.1 days for calves treated with a bolus of multi-strain probiotic versus 5.9 days for the control group. In a supplementation with oregano essential oil, calves receiving the treatment recovered in 3.8 days versus 5.2 days for the control group (Panagiotis et al., 2017). Finally, Ridell et al. (2010) described no difference in the time with diarrhea of calves fed a *Bacillus*-based probiotic and control calves, 4.65 vs 5.45 days. In these studies, the biological or economic relevance of the recovery time was not assessed.

The information related to recovery for the first diarrhea episode is suggesting a problem with chronic diarrhea in the facility, not associated with the treatment. The survival curve showed that 50% of the calves recovered in over 7 days. Moreover, it is important to mention that around 20% of all enrolled calves recovered between 15 and 30 days.

In this study, the number of new diarrhea events during the study period ranged between 0 and 4 by calf and between 1 and 6 new events by pair. It is important to clarify that some calves had multiple short-lasting events, while other calves had 1 or fewer long-lasting events. Therefore, for this study, fewer events might not represent enhanced health.

A normal consistency in feces is the consequence of an adequate state of the intestinal mucosa that results in good absorption of nutrients (Signorini et al., 2020). In our study, the rate of severe diarrhea events was not associated with the administration of probiotics in colostrum and milk. In agreement with our results, Signorini et al. (2012), in a meta-analysis, found no association between fecal consistency and the addition of probiotics in the diet of pre-weaned dairy calves. On the other hand, twelve studies were compared for fecal consistency in a systematic review. The authors found a tendency for a lower fecal score of calves supplemented with probiotics (Alawneh et al., 2020). Moreover, a trial using milk replacer supplemented with *Lactobacillus* found a reduction in the morbidity, severity, and mortality associated with diarrhea caused by Rotavirus (Kayasaki et al., 2021).

Performance

The addition of a multi-strain probiotic in colostrum and milk of pre-weaned calves was not associated with differences in ADG. Published studies are not conclusive in the benefits of probiotic supplementation in the growth and performance of young dairy calves (Zhang et al., 2015; Cantor et al., 2019; Cangiano et al., 2020). It has been described that calves receiving probiotics had on average a greater ADG in the first 3 weeks of life (Cantor et al., 2019; Alawneh et al., 2020). In our study, no differences were observed in the ADG calculated for the first 30 days of life.

Furthermore, there is a wide range of values for ADG in pre-weaned calves supplemented with bacterial-based probiotic. ADG had been reported from 280 to 770gr/d (Sun et al., 2010; Ridell et al. 2010; Frizzo et al., 2011; Zhang et al., 2015; Foditsh et al., 2015; Stefanska et al., 2021; Salazar et al., 2019). Therefore, even if there is a significant difference in ADG, this one might not represent optimal growth for the pre-weaned period.

A well-known benchmark for dairy calf performance is to double the birth weight at weaning, which is in average 9 weeks (DCHA, 2016). The mean weight gain for our treatment groups showed that calves did not reach this goal as a group. Moreover, a new categorization for the ADG in the pre-weaned period has been suggested as follows: Excellent>0.82 kg/d; Fair 0.64-0.82 kg/d; and Poor <0.64 kg/d (Shivley et al., 2018). Comparing our results to the proposed categories, the mean ADG for the pre-weaned period was poor for CTR and PB calves. Perhaps, high THI, chronicity of diarrhea, and potential malabsorption syndrome might be the causes leading to poor weight gain.

Culling

Overall, 41 calves left the facility during the study time. As this is an organic-certified facility, calves that were administered or required antibiotic therapy were sold. A smaller number of animals were euthanized in the facility. The overall mortality for the study time was 10.64%, which was higher than the 5.0% reported as the national average (Urie et al., 2018). The main cause of death was digestive problems, followed by respiratory disease. The majority of the calves that died due to diarrhea were born in 3 consecutive days, from August 4th to 6th, 2020. When the week of birth/enrollment was analyzed as a potential confounder, it was not significant for the odds of culling. However, during week 3 of enrollment, an increased calving rate was observed. Therefore, a greater number of animals were moved from the maternity to the rearing facility. Perhaps, this increase in the number of newborn calves might have impacted management practices.

Fecal screen

The administration of probiotics to pre-weaned dairy calves has shown some positive effects by reducing the severity and counts of *Salmonella* and *E.Coli* in induced diarrhea (Zhao et al., 2003; Roodposhti and Dabiri, 2012; Liang et al., 2020). In our study, fecal samples were collected at 3 times from a subsample of calves for a diarrhea screen. The diarrhea screen included bacteria, viruses, and parasites. The most prevalent pathogen in the study was *Clostridium,* where CTR calves tending to have a positive exam when compared to PB calves. Consistent with our results, a systematic review found a tendency for the effect of bacterial-based probiotics on reduction of Clostridium (Alawhen et al., 2020). Additionally, no difference was observed in Cryptosporidium detection by treatment groups. Similarly, the prophylactic administration of a lactic acid probiotic did not protect pre-weaned calves from the infection with *Cryptosporidium parvum* in field conditions (Harp et al., 1996). *Salmonella*, Rotavirus, and Coronavirus were found in few samples at the 3 sample points. BVDV was not detected in any sample.

Serum Haptoglobin

Haptoglobin is an acute-phase protein (APP) that has been associated with inflammation and infection in cattle (Thóthová et al., 2013; Murray et al., 2014). This APP has been identified as a good biomarker for the evaluation of health in dairy calves (Seppa-Lassila et al., 2013).

Murray et al. (2014) found that serum Hp $\geq$ 0.13g/L during the first week of life was associated with increased morbidity and mortality in calves up to 4 months of age. In our study, we observed a significant difference in serum Hp concentration at sampling 1 (3d of life). Control calves had a higher concentration of serum Hp when compared to PB calves. No differences were found in the presentation of diarrhea or culling by treatment group during the study period.

In several studies, serum Hp concentration up to 200 ug/mL had been described for healthy cattle, including calves (Chua et al., 2004; Seppa-Lassila et al., 2013; Niekerk et al., 2021; Hanthorn et al., 2014). In this study, the mean serum Hp was over 200 ug/ml at sampling point 2, 3, and 7. Statistical differences were found only at sampling point 2, where treatment calves had a greater concentration of serum Hp. Sampling point 2 and 3 (day 7 and 14 of life) were related with the onset of diarrhea in the enrolled calves. Therefore, it is likely that some of the sampled animals were presenting an increase in Hp due to early infection (Seppa-Lassila et el., 2013, Thóthová et al., 2013).

On the other hand, sampling point 7 was around day 50 of life. In the survival curve for culling, it is possible to observe that between day 40 and 50 of life, several animals left the facility. According to our records, some calves experienced clinical BRD at that age (data not shown). Therefore, the inflammatory process due to respiratory disease might explain the increase in serum Hp concentration at that sampling point.

Lung ultrasound

Lung ultrasound is a diagnostic tool that is gaining attention for its use on farms for the detection and diagnostic of BRD (Ollivet and Buczinski, 2016). Respiratory diseases are the second most common cause of morbidity and death in pre-weaned calves (USDA, 2011; Urie et al., 2018). During the study period, a similar number of calves were detected with subclinical BRD by treatment group. Therefore, the treatment did not improve the overall health of the calves.

Milk intake

Finally, we collected information related to the milk intake from all calves. The intake was estimated only in the morning feedings of days when the probiotic was mixed in the milk. The objective of doing this was to detect potential refusal of the milk due to the treatment. We do not have enough data to conclude that treatment calves consumed more milk. Regardless, PB calves consume consistently over 95% of the offered milk, CTR calves consumed over 89% of the milk provided. Therefore, milk refusal was not observed due to the addition of probiotics in milk.

As mentioned through the discussion, there is no consistent evidence in the peer review literature to suggest, probiotics might have a positive effect on pre-weaned calf health and performance. An important and unexcepted variable was the THI in the study period. Heat stress might have impaired the health of calves and masked a potential effect of the probiotic intervention. Additionally, in this study, the PBF2 was administered 3 times per week, as indicated by the manufacturer recommendations. In most of the peer-reviewed studies, a daily dose was administered to calves when probiotics were used as a prophylactic strategy. Moreover, the probiotic used in this study was refrigerated after opening. Although, the viability of probiotics once mixed with the colostrum or milk was not assessed.

Finally, this study was performed during the COVID-19 pandemic. In the study period, outbreaks were common in Northern Colorado, and vaccines were not available. It is possible that the mental health of caretakers might have been affected during these uncertain times, potentially affecting their work routines and performance. Although, we do not have data to suggest this is correct.

Conclusion

The provision of a bacterial-based probiotic in colostrum and milk did not impact the health and performance of pre-weaned dairy calves. No detrimental effects were observed due to the use of these products.

Tables and Figures

Table 3.1: Probiotic formulations provided to newborn and pre-weaned calves.

Probiotic Formulation 1[a]		Probiotic Formulation 2[b]	
Probiotic bacteria	Concentration (CFU/dose)	Probiotic bacteria	Concentration (CFU/dose)
Enterococcus faecium	2 Billion	*Enterococcus faecium*	1 Billion
Lactobacillus casei KE01	2 Billion	*Lactobacillus casei* KE01 Supernantant	100 mg/dose
Bifidobacterium longum	500 Million	*Bacillus sutilis*	2 Billion
Bifidobacterium bifidium	500 Million	*Bacillus licheniformis*	1 Billion
Lactobacillus acidophilus	1 Billion	Carrier organic tapioca	
Carrier organic tapioca			

[a]Establish Newborn-O: 2gr mixed with colostrum
[b]Establish Young-O: 1gr mixed with milk

Table 3.2: Scoring system used to assess health status in the enrolled calves (modified from Wattiaux, 2005; McGuirk, 2008b; Smith 2009; and Perino and Apley, 1995)

Health variable	Score 0	1	2	3
Diarrhea	Normal	Semi-formed, pasty	Loose, but stays on top of bedding	Watery, sifts through bedding
Ear Position	Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
Nasal discharge	Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
Ocular discharge	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
Cough	None	Spontaneous cough	-	-
Attitude	Normal, no signs of depression, vigorous suckling	Mild depression, no signs of weakness, actively follows movements, suckle but not vigorously	Moderate depression, slight altered gait, able to stand, moves slower than pen mate, weak suckle	Severe depression with incoordination, altered gait, unable to stand or suckle
Dehydration	Normal, eyes are bright, no eyeball recession, skin tent <1s	Mild, eyeball recession 2-4mm, skin tent <3s	Moderate, eyeball recession 4-5mm, skin tent 3-5s	Severe, eyeball recession >6mm, skin tent >5

Table 3.3: LSM for serum total protein (STP) and performance parameters by treatment group (CTR=control, PB= probiotic). Significance level <0.05.

	CTR	PB	
Variable	LMS±SE	LMS±SE	P-value
STP (g/dL)[a]	6.75±0.06	6.58±0.06	0.06
Birth weight (Kg)[a]	39.2±0.17	38.7±0.17	0.04
Weight 30d (Kg)[b]	52.7±0.56	51.8±0.57	0.26
Weaning weight (Kg)[c]	75.7±0.99	74.6±0.98	0.46
ADG 30d(gr/d)[b]	437.7±22.95	445.4±23.59	0.71
ADG weaning(gr/d)[c]	562.5±13.92	570.8±13.53	0.67

[a]n=232 (CTR=116, PB=116)
[b]n= 211 (CTR=108, PB=103)
[c]n=189 (CTR=93, PB=96)

Table 3.4: Comparison of diarrhea and culling outcomes organized by statistical analysis and treatment group (CTR=control, PB= probiotic). Significance level P<0.05.

Analysis Time to Event	CTR median time (95%CI)	PB median time (95%CI)	P-value
First diarrhea (d)	11 (10-12)	11 (10-12)	0.97
Recovery first diarrhea(d)	7(5-7)	7(5-9)	0.41
Culling(d)[a]	51.11±0.98	48.37±1.11	0.94
Proportional Hazard Ratio (CTR vs PB)	Hazard ratio	95%CI	
First diarrhea	1.05	0.82-1.36	0.69
Recovery first diarrhea	1.07	0.81-1.42	0.63
Logistic Regression (CTR vs PB)	OR	95%CI	
Other signs of disease[b]	1.4	0.69-2.92	0.33
Severe diarrhea[c]	0.82	0.45-1.48	0.50
Culling	1.06	0.45-2.55	0.88
Poisson Regression (CTR vs PB)	Rate	95%CI	
Total diarrhea events	1.01	0.89-1.15	0.86
Severe diarrhea exams[d]	0.93	0.72-1.23	0.63

[a]Culling: dead, sold, and euthanized animals
[b]Other signs of disease in the first diarrhea event (dehydration and/or abnormal attitude)
[c]Severe diarrhea: fecal score 3 at any point in the study period
[d]Severe diarrhea exams: number of total clinical examinations with fecal score 3

Table 3.5: Number of calves positive to fecal pathogens at 3 sampling points screening.

	Sample Number				
Pathogen	1[a]	2[b]	3[c]	Total	*P*-value[d]
Clostridium					
CTR, n	20	18	12	50	0.06
PB, n	17	10	10	37	
Cryptosporidium					
CTR, n	6	21	2	29	0.11
PB, n	11	20	2	33	
Salmonella					
CTR, n	0	0	0	0	-
PB, n	2	1	2	5	
Coronavirus					
CTR, n	0	0	0	0	-
PB, n	0	1	0	1	
Rotavirus					
CTR, n	0	2	5	7	-
PB, n	2	0	7	9	
BVDV					
CTR, n	0	0	0	0	-
PB, n	0	0	0	0	

[a] Day 7 of life, n=50 (CTR=25, PB=25)
[b] Day 14 of life, n=47 (CTR=25, PB=22)
[c] Day 21 of life, n=43 (CTR=22, PB=21)
[d]P-value for the repeated measure analysis for binary response.

Table 3.6: Results of weekly lung ultrasound, represented as the number (%) of calves positive to lung consolidation (≥1cm^2) in absence of respiratory clinical signs by treatment group (CTR=control, PB= probiotic).

	Ultrasound exam							
Treatment group	1	2	3	4	5	6	7	8
CTR, n(%)	0 (0)	1 (10)	2 (20)	2 (20)	5 (50)	5 (50)	5 (50)	4 (44.4)
PB, n(%)	0 (0)	0 (0)	0 (0)	3 (30)	5 (50)	4 (44.4)	4 (44.4)	4 (44.4)
N(CTR, PB)[a]	10,10	10,10	10,10	10,10	10,10	10,9	10,9	9,9

[a]Total number of calves examined by treatment group

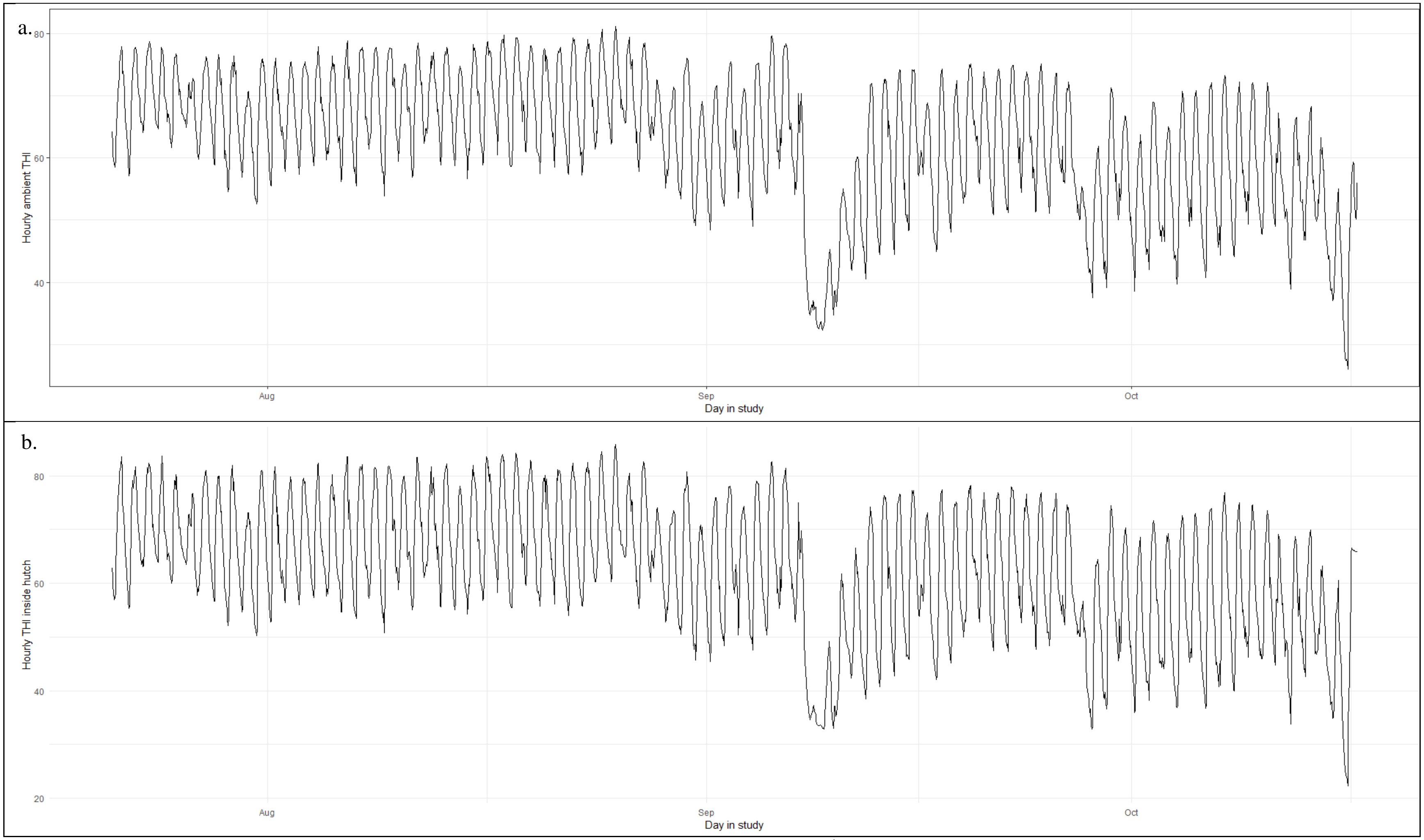

Figure 3.1: Hourly THI for the complete study period (July 21st to October 16th, 2020). a) Ambient THI. b) THI inside the hutch.

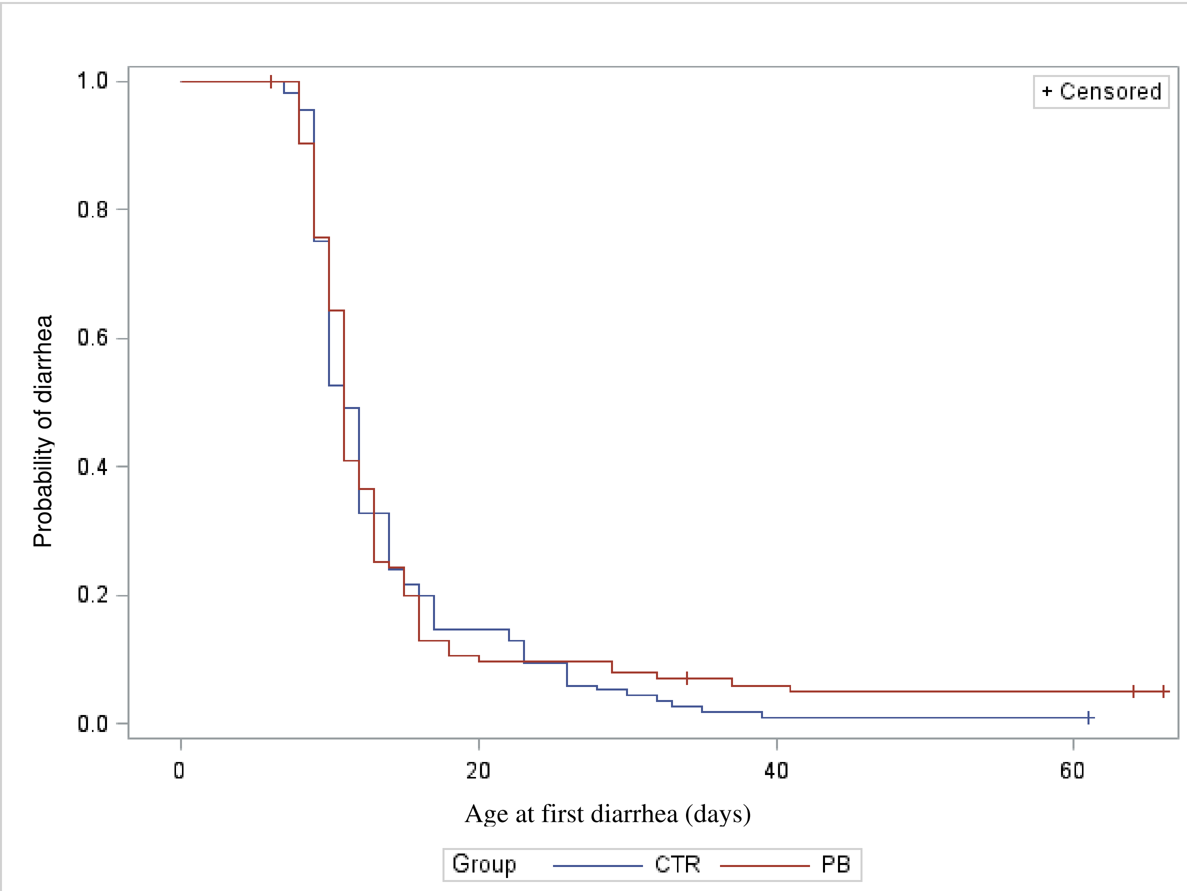

Figure 3.2: Time to occurrence of the first diarrhea event. Control (CTR=116 calves; blue line), Probiotic (PB=116 calves; red line). *P*=0.97

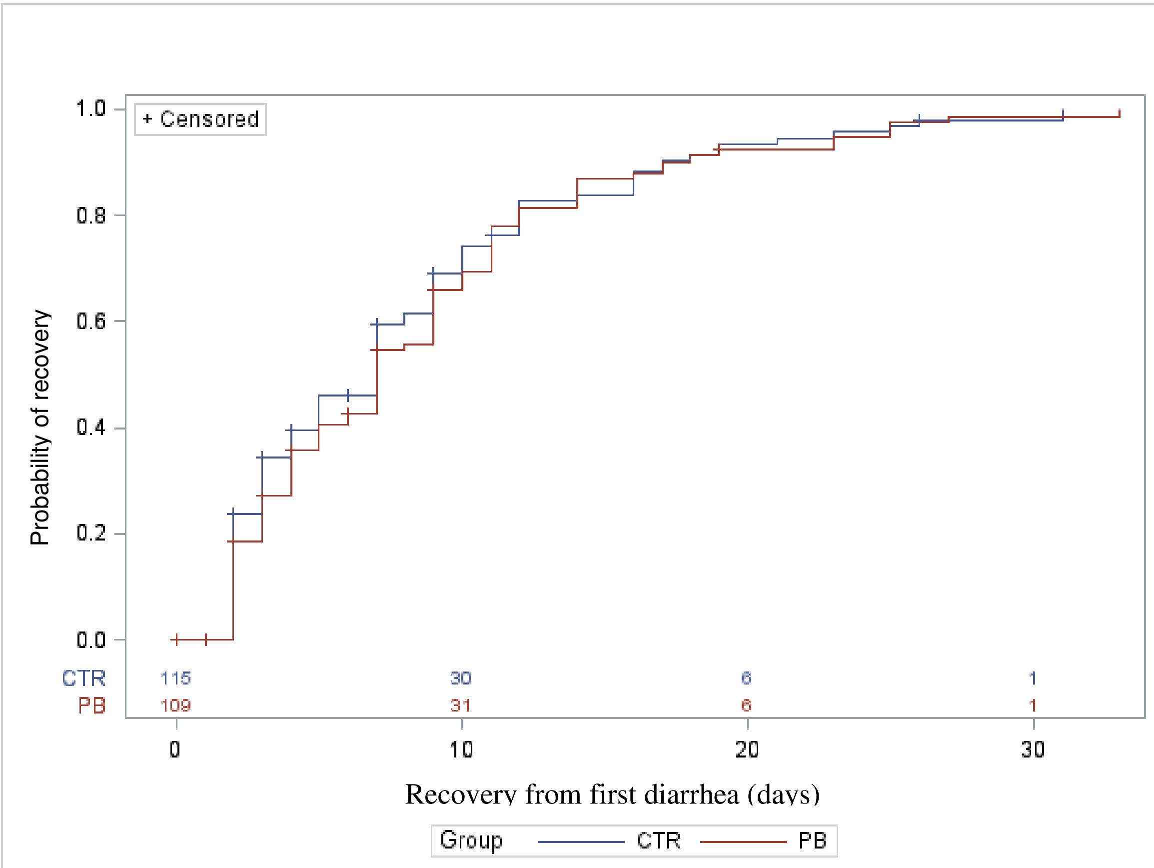

Figure 3.3: Time to recovery from the first diarrhea event. Control (CTR=115 calves; blue line), Probiotic (PB=109 calves; red line). *P*=0.41

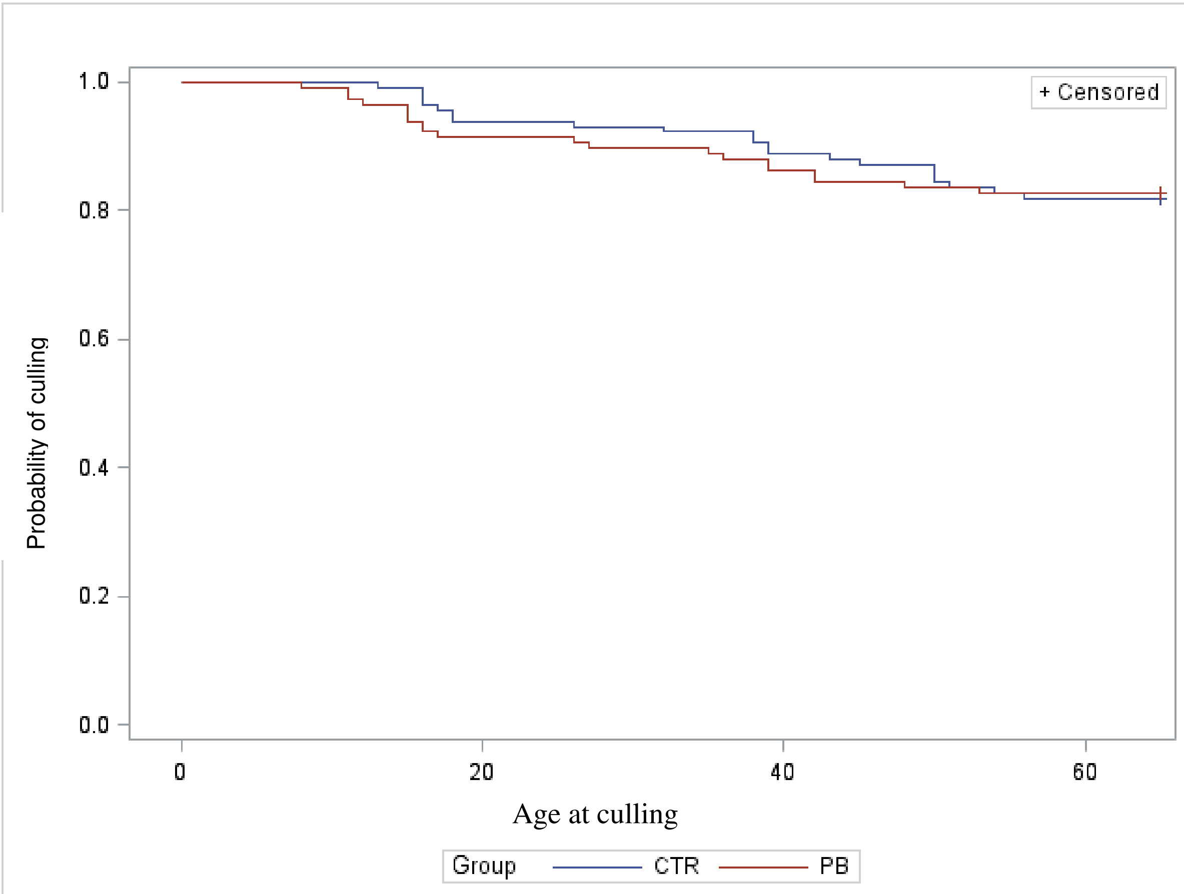

Figure 3.4: Time to culling during the study period (birth to weaning). Control (CTR=116 calves; blue line), Probiotic (PB=116 calves; red line). *P*=0.94

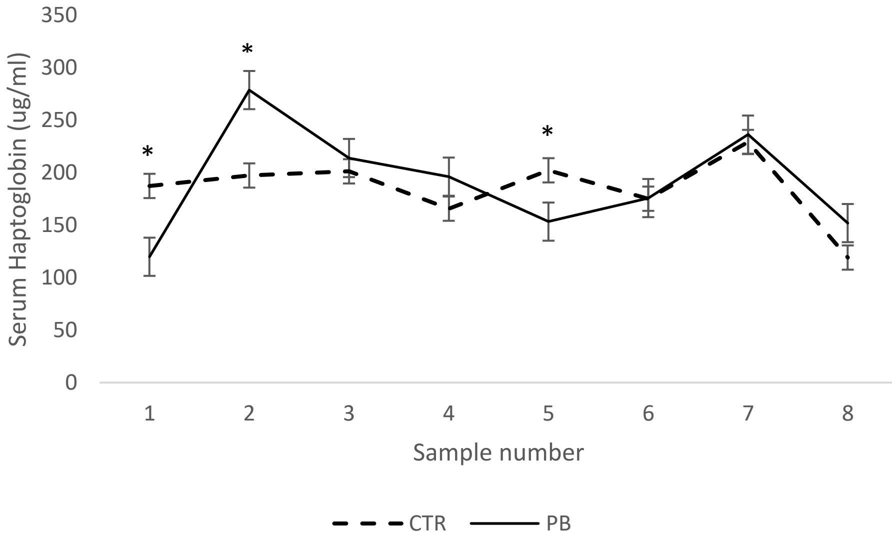

Figure 3.5: Repeated measures analysis for serum Haptoglobin concentration (ug/ml) at 8 sample points from day 3 of life to weaning. Interaction term for treatment group (control=CTR, dashed line; probiotic=PB, solid line) and sample number *P*=0.02. Asterisks indicate significant differences.

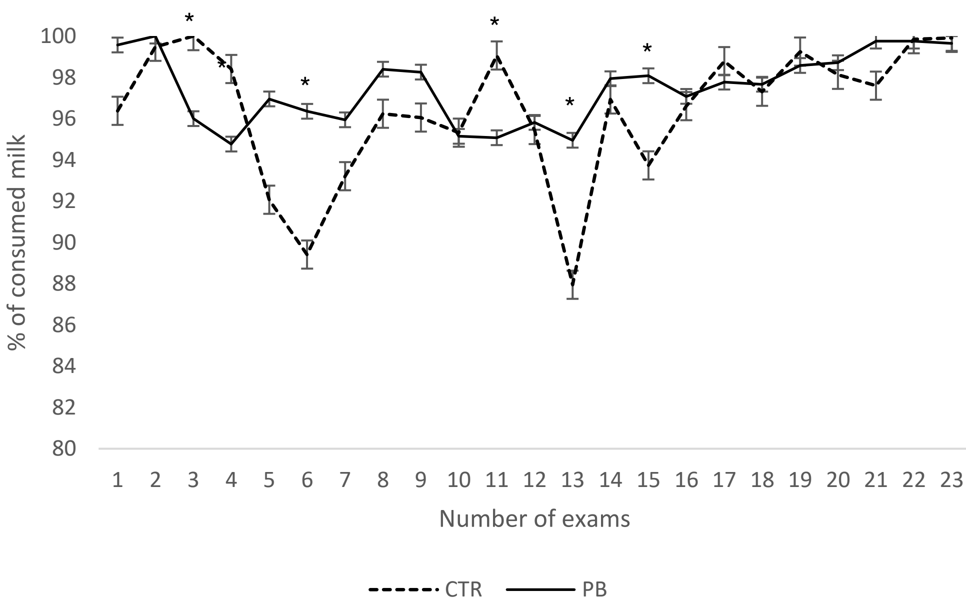

Figure 3.6: Repeated measures analysis for milk consumption (%) per health exam during the study period. Interaction term for treatment group (control=CTR, dashed line; probiotic=PB, solid line) and exam number *P*=0.001. Asterisks indicate significant differences.

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CHAPTER 4: DESCRIPTION OF EARLY LIFE BEHAVIOR AND DISEASE DYNAMICS OF PAIRED HOUSED HOLSTEIN HEIFER CALVES

Introduction

The separation of the newborn calf from the dam soon after birth is a common practice in modern dairy farms. In the US, only 13.4% of the calves are housed in groups, while individual housing is the most common management strategy for the pre-weaned period (Urie et al., 2018a). The justification for individual housing is the prevention of disease transmission, improved performance, and the reduction of agonist behaviors, such as cross-sucking (Pempek et al., 2013).

In natural or seminatural conditions, calves are part of a herd, where their interactions with adult and young cattle reflect their social nature (Vitale et al., 1986). In recent years, increasing and robust evidence demonstrates the benefits of social housing for young dairy calves and the detrimental effects of social isolation early in life (Jensen et al., 1998; Chua et al., 2002; De Paula Vieira, 2010; Whalin et al. 2018).

When calves are pair housed from birth or within the first 3 weeks of life, they establish a strong bond with each other (Færevik et al., 2007;_Duve and Jensen; 2011). Furthermore, in a conditioning study by Holm et al. (2002) that tested calves' preferences for contact with a conspecific, calves' motivation for full social companion was stronger than for partial contact.

Scientific evidence suggests that providing social companion to pre-weaned calves increases dry matter intake and ADG (De Paula Vieira, 2010; Costa et al., 2015; Jensen et al. 2015; Pempek et al., 2016), potentially due to social facilitation or social learning (Costa et al., 2015). Additionally, pair housed calves exposed to a novel food, are less fearful and consumed more than individually housed calves (Whalin et al. 2018). Moreover, after weaning, pair housed calves had a shorter latency to start feeding and spent more time in the feed bunk than individually housed calves (De Paula Vieira et al., 2010).

Behavior is also impacted by pair housing. Calves with a companion have the opportunity to perform social behavior and social play behavior, which are indicators of good welfare (Jensen et al., 1998; Duve and Jensen, 2012). Additionally, pair housed pre-weaned calves are more active (Jensen et al., 1998) and vocalize less at weaning (De Paula Vieira et al., 2010; Bolt et al., 2017).

As reported by Gaillard et al. (2014), social housing improves cognitive performance in calves. These authors compared individual and paired calves' responses in 2 cognitive tests, reversal learning through color discrimination and novel object recognition. Pair housed calves were able to adapt more quickly to the reversal task, and they familiarized themselves faster with the novel object than individual housed calves.

When pair housed calves are provided adequate amounts of milk or milk replacer, agonistic behaviors or competition at feeding time are rarely observed (Chua et al., 2002; Wormsbecher et al., 2017; Whalin et al., 2018). On the contrary, in calves housed in pairs with low milk allowance, agonistic behaviors are frequent, and play behavior is reduced (Jensen et al., 2008; Jensen et al., 2015; Pempek et al., 2016).

All this is valuable information; however, little is known about calves' interaction and behavioral dynamics in their first days of life, when they are housed in pairs since birth. Additionally, the presentation of diseases within pair have not been extensively studied. Therefore, this observational study had 2 objectives. First, to describe the behavioral dynamics and interactions in the first 10 days of pair housing. Second, to describe the presentation of diseases within pair in the first 30 days of life. The behaviors of interest in this study were resting and active time, play behavior, time together, and the presentation of agonistic behaviors at the feeding time. We hypothesized that calves would modify their time budget for activities and time together in the first days of pair housing.

Material and Methods

The study was performed in accordance with the guidelines set by Colorado State University Institutional Animal Care and Use Committee (protocol ID: 985).

Experimental design, animals, and management practices

This observational study was conducted in an organic-certified dairy calf rearing facility in Northern Colorado from July 22nd to September 11th, 2020. One hundred and sixteen Holstein heifer calves (58 pairs) were enrolled at birth and followed for the first 30 days of life for disease dynamic description. A subsample (n=30) was followed for the first 10 days of life for behavioral description. The enrolled calves were the control calves from Chapter 3.

Detailed management of calves was described in the chapter "Evaluation of a probiotic program on health and performance of organic certified Holstein heifer calves." Briefly, calves were immediately separated from the dam at birth and moved into a newborn pen. Calves were offered 4L of high-quality colostrum (≥52 mg/ml immunoglobulins) within the first hour of life. In the 2nd and 3rd feeding, calves were provided 2L of high-quality colostrum at 12 and 24 hours of life. Colostrum quality was measured with a colostrometer (Biogenics, Florence, OR).

Once calves were moved to the rearing facility, they were housed in pairs by following the farm ID number. The calf unit consisted of 2 individual polyethylene calf hutches (Agri-Plastics, Stoney Creek, ON, Canada) enclosed by a wire fence in a total area of $4.5m^2$. Two bottle holders were placed in each corner of the enclosing fence. An 86 cm wire wall delimited the milk feeding area and functioned as a physical barrier at feeding time. Additionally, two buckets for water and two buckets for organic certified calf starter (16% Organic Calf Starter, Feedex Companies, LLC. South Hutchinsin, KS) were positioned in the front of the enclosing gate. Fifty calf units, separated by 1 meter of distance, conformed a row of calves at this facility.

Pre-weaned calves were provided three milk feedings per day at 6:30 AM, 1:00 PM, and 7:30 PM. Pasteurized whole milk was offered to the calves in nursing bottles. The milk allowance in the first month increased from 5.6 L in the first week to 10.4 L for weeks 2, 3, and 4 of life. Clean and fresh water was offered since the arrival to the facility, and calf starter was provided at day 4 of life.

Daily health assessments and treatments were performed by trained caretakers following the farm SOP. As this is a certified organic farm, animals needing antibiotic therapy were sold to another rearing facility.

Data collection

As this study was performed in Summer (July 22nd to September 10th, 2020), temperature and humidity were expected to be a stressing factor. Therefore, the temperature and humidity inside the hutch were measured with the use of 2 HOBO UX100-011 temp/RH 2.5% loggers (Onset Computer Corporation., Bourne, MA). The loggers were mounted in the ceiling of an empty hutch located in the same area where the calves were housed. The devices were set to record temperature and humidity every 30 minutes for the study period. For the calculation of the temperature-humidity index (THI) the following equation was used: THI = (1.8 x T + 32) – ((0.55 – 0.0055 x RH) x (1.8 x T – 26)), where T = temperature (°C) and RH = relative humidity (Kendall et al., 2008; Vickers et al., 2010).

The transfer of passive immunity was assessed as a baseline measurement for all calves. A blood sample of 5 to 10 ml was collected to measure serum total protein (STP) concentration from all the enrolled calves. Blood was obtained via jugular venipuncture using a vacutainer and a tube without anticoagulant (Becton Dickinson Vacutainer, Franklin Lakes, NJ). After collection, samples were allowed to clot and centrifuged at 2,200 rpm for 15 minutes. The quantification of STP was performed with the use of a digital refractometer (PA200-013 Palm Abbe, Misco Refractometer, Solon, OH).

Behavior in the first 10 days of pair housing was observed from a subgroup of calves (n=30, pairs=15). In total, 4 time-lapse video cameras (Brinno TLC200 Pro, Stuart, FL) were set to record 2 to 3 calf units every 10 seconds. A pre-test was made to determine the most appropriate time-lapse for the recording. The cameras were placed in front of the hutches, at an angle that allowed visibility of the outside yard and movement inside the hutch. Calves were observed daily in 3 time periods (AM, Noon, PM). Each period corresponded to 120 minutes of observation, 60 minutes before and 60 minutes after the milk feeding. In total, pairs were observed for 360 minutes/day, and observations were averaged by pair.

The behaviors observed from this group of calves are defined in Table 4.1 (Jensen et al., 2008; Duve and Jensen, 2012; Pempek et al., 2016). For the period "before and after feeding time", active time, resting time, locomotor play, locomotor synchronized play, social play, time as pair inside the hutch, time as pair outside the hutch, and time alone were measured as min/120 minutes per period of observation. Additionally, binary observations were collected for each day and period at feeding time. These behaviors included contacting, displacing, and cross-sucking (Table 4.1). Finally, a daily observation was added for water and starter intake, along with the provision of training or assistance at the milk feeding (Table 4.1). As calves were transferred to the rearing facility in the afternoon of day 1 of life, information regarding AM and Noon period was not collected. Due to technical problems, the recording of 3 pairs of calves was not complete for the 10 days of observation.

For the estimation of average daily gain (ADG) in the study period, heart girth circumference of calves was measured at birth and day 30 of life with the Holstein Calf Weight (The Coburn Company Inc., Whitewater, WI). One researcher measured all enrolled calves. A clinical examination was performed three times per week on all the enrolled calves for the entire study period. Health was evaluated with a modified version of the Wisconsin Calf Health Chart (McGuirk, 2008), dehydration score (Wattiaux, 2005; Smith, 2009), and attitude score (Perino and Apley, 1995). All the scores used were as a 4-point scale (Table 4.2). Scores 0 and 1 were considered normal, and scores 2 and 3 were considered abnormal. One researcher performed all the clinical examinations.

In addition, a severity score was created by combining the records obtained in the clinical examination. The description for healthy, mild, moderate, and severe status is provided in Table 4.3. For analysis, severity was dichotomized as healthy (healthy and mild); moderate (moderate and severe). Finally, dates and causes for dead and sold animals were recorded.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Data were organized in Microsoft Excel for Microsoft 365 (Microsoft Corporation, Redmond, WA). For behavioral variables, the experimental unit was the pair of calves.

Data corresponding to THI was organized in Microsoft Excel. Ambient and hutch THI were averaged and graph by period and day in observation. PROC MEANS was performed for variable description (mean, median, range, and SE) of THI, STP concentration, birth weight, age at death, weight at 30 days of life, and ADG.

Normality was assessed with PROC UNIVARIATE using the Shapiro Wilk test for continuous behavioral variables (resting and active time, time alone, and time together). Homogeneity of variance was evaluated with PROC GLM using Levene's Test. Behavioral variables that were not normally distributed were square-root transformed for analysis. A general linear model using PROC MIXED was performed to detect differences in the time budget of behavioral variables within periods (AM, Noon, PM) and days in observation. Pair number was used as the repeated statement and day of life as the predictor variable. Play behavior (locomotor play, locomotor synchronized play, and social play) was not normalized with the square root transformation, and variance was unequal for the Noon period. Therefore, it was described as the percentage of the active time that calves performed each play behavior by period and day in observation.

In addition, all binary behaviors and daily behaviors were described with PROC FREQ for each period in observation; the Chi-Square test was used to determine statistical significance. In addition, PROC FREQ was used to describe health outcomes and mortality. Moreover, PROC GENMOD was used to determine the likelihood of a pair of calves being together or alone at the beginning of the period. Pair number was used as the repeated statement and day in observation as the predictor variable.

Results

Weather conditions

A detailed description of THI (July 22nd to September 10th, 2020) is available in A3.1. The mean (±SD) THI during the study period was 66.4±7.4 units (range: 32.9 - 85.9 units). During the day hours (8 AM to 7 PM), the mean (±SD) THI for the entire study period was 73.4±8.4 units, with a median of 76.0 units. In the night hours (8 PM to 7 AM), the mean (±SD) THI was 59.3±6.7 units, with a median of 61.1 units.

The mean THI was calculated for the periods observed (AM, Noon, and PM) in the first 10 days of life of the calves. In the secondary axis of Figure 4.1a,b,c is presented a graphical representation of the mean THI by period and day of observation. The period with the lower THI was the AM; calves were exposed to a mean (±SD) THI inside the hutch of 60.4±2.5 units (range: 59.2 - 61.3 units). The period with the greatest THI was Noon, with a mean (±SD) of 80.6±2.1 units (range: 79.3 to 81.4 units). Finally, the mean (±SD) THI for the PM period was 71.3±1.65 (range: 69.5 and 72.3).

Baseline measurements

The calves enrolled in this observational study had a mean STP (±SE) concentration of 6.75±0.06 g/dL (range: 5.2-9.2 g/dl; Table 4.4). When considering the most recent recommendations for passive immunity in dairy calves (Lombard et al., 2020), 80.2% (n=93) of the calves were in the Excellent category, 10.3% (n=12) of the calves were in the Good category, and 9.5% (n=18) of the calves were in the Fair category. The mean (±SE) birthweight was 39.18±0.18 kg (range: 32.3 - 44.1 kg; Table 4.4).

Behavioral time budget

Resting time changed across days of life for the AM (P<0.0001; Figure 4.1a) and PM (P<0.0001; Figure 4.1c) periods. The mean (±SE) minutes spent resting ranged for the AM (75.5±2.8 to 102.5±2.8 min), Noon (95.9±3.4 to 107.9±3.4 min), and PM (75.8±4.1 to 106.6±3.4 min) periods. Likewise, active time varied for the AM (P<0.0001; Figure 4.1a) and PM (P<0.0001; Figure 4.1c) periods in the study time. The mean (±SE) minutes spent active ranged for the AM (17.4±2.8 to 44.5±2.3 min), Noon (12.1±3.4 to 24.1±3.4 min), and PM (13.3±3.6 to 44.1±4.1 min) periods. Lastly, in the Noon period, no difference was observed in resting or active time in the first 10 days of pair housing (Figure 4.1b). Overall, on days 1 and 2, pairs spent the longest time resting and the shortest time active (Figure 4.1a,b,c).

The mean percentage (±SE) of the active time that calves spent performing any play behavior ranged for AM (52.3±5.4 to 84.5±5.2 %), Noon (15.6±6.9 to 49.2±6.4%.), and PM (62.4±4.1 to 81.9±6.8%) periods. Interestingly, calves spent a greater percentage of their active time interacting as synchronized and social play behavior rather than locomotor play. It was observed from day 3 in the AM (Figure 4.2a), from day 4 in the Noon period (Figure 4.2b), and from day 2 in the PM period (Figure 4.2c). An exception was day 10 at Noon, where calves did not spend any percentage of the active time performing social play behavior (Figure 4.2b).

In relation to the time calves spent alone by period, significant changes across days of life were determined for the AM period (P<0.0001; Figure 4.3a), and no difference for the Noon period (P=0.31; Figure 4.3b) and PM period (P=0.60; Figure 4.3c). In the AM period, calves decreased their time alone (mean±SE) from 95.3±9.3 min to 53.1±8.5 min, from day 2 to 10 of life. The mean (±SE) of time that calves spent alone ranged for the Noon (47.5 ±11.3 to 80.4±12.8min) and PM (48.1±9.6 to 86.4±9.6 min). However, the difference did not follow a trend over time.

Similarly, the time calves spent together inside the hutch varied by day for the AM period (P=0.003; Figure 4.3a). No differences were observed for the Noon (P=0.41; Figure 4.3b) and PM (0.51; Figure 4.3c) periods. In the AM period, calves increased their time together inside the hutch (mean±SE) from 17.9±8.7 to 46.7±7.9 min from day 2 to day 10 in observation. The mean (±SE) of time that calves spent together inside the hutch ranged for the Noon (36.1±11.2 to 65.88±11.2 min) and PM (29.6±9.1 to 47.4±9.1). However, the difference did not follow a trend over time.

Finally, significant variation across time was found for the time calves spent together outside the hutch in the AM (P=0.0001; Figure 4.3a) and PM periods (P=0.004; Figure 4.3c). No difference was observed for the Noon period (P=0.21; Figure 4.3b). In the AM period, calves increased their time together outside the hutch (mean±SE) from 7.3±3.6 to 20.3±3.3 min, for days 2 to 10. While in the PM period, the increase was from 3.6±4.9 to 27.4±5.5 min for days 1 to 10. Interestingly, the mean time (±SE) calves spent together outside the hutch in the Noon period ranged between 1.2±1.9 to 6.5±1.9 min/120min (Figure 4.3b).

When combining the time inside and outside the hutch, calves spent more time together than alone from day 6 in the AM period. At Noon, calves spent close to the same amount of time alone or together starting at day 4, although their time together was preferably inside the hutch. And in the PM period, calves spent more time together from day 3 in observation (Figure 4.3a,b,c).

Binary behaviors

Calves were monitored at the start of each observation period to determine if they were together or separate. Overall, calves were found together in the AM, Noon, and PM period a 50% (P=0.25), 42.7% (P=0.37), and 41% (P=0.71) of the times, respectively (Table 4.5). Additionally, no difference was found in the likelihood of a pair of being together or alone when adjusting for day in observation for the AM (OR: 0.47, 95% CI; 0.11-2.04; P=0.31), Noon (OR: 1.6, 95% CI: 0.18-14.7; P=0.65), and PM (OR: 0.46, 95% CI: 0.08-2.9; P=0.41) periods.

For the AM period, the minimum number of calves together was in the morning of day 2 (n=4; 26.6%). However, from day 6 to 10 of life, 9 (60%) pairs of calves were consistently together. In the Noon period, a greater number of pairs were found separate on days 2, 3, 4, 7, 8, and 10. The minimum number of pairs together was 3 (20%) on day 3, and the maximum number of pairs together was 9 (60%) on day 9 in observation. A similar situation was observed in the PM period; calves were found in a greater percentage alone at days 2, 3, 4, 8, 9, and 10. The minimum number of pairs together was 4 (26.6%) on day 1, and the maximum number of calves together was 8 (53.0%) on day 6 of life.

Overall, from the days observed, the percentage of pairs contacting at feeding time was 26.2% (P<0.0001), 18.3% (P<0.0001), and 25.0% (P<0.0001) for the AM, Noon, and PM period, respectively (Table 4.5). Contacting was not observed in all periods; it was not detected at days 8 and 9 in the AM and Noon periods or at days 1, 7, and 10 in the PM period. The maximum number of calves contacting the pen mate was 6, representing 40% of the observed pairs. This interaction occurred on day 3 in the AM, Noon, and PM period, on day 4 in the PM period, and on day 6 in the AM period.

In addition, calves were observed for displacing behavior at feeding time. This behavior was less frequent than contacting. The percentage of pairs displacing was 10.3% (P<0.0001), 5.6% (P<0.0001), and 11.2% (P<0.0001) for the AM, Noon and PM period, respectively (Table 4.5). Displacements were not observed at days 2, 8, and 10 in the AM period; at days 1, 2, 8, and 9 in the Noon period; and at days 1, 7, and 10 in the PM period. The maximum number of pairs that demonstrated displacing behavior was 4 (26.6%) on day 4 in the AM period and on day 6 in the PM period. At Noon only one pair (6.7%) exhibited this behavior.

Cross-sucking was observed in a low frequency throughout the study period. For each period in observation, the percentage of calves performing this behavior was 7.9% ($P<0.0001$), 3.2% ($P<0.0001$), and 4.2% ($P<0.0001$) in the AM, Noon, and PM period, respectively (Table 4.5). Cross sucking was not performed on day 2, 7, 8 in AM period, on day 1, 2, 3, 5, 8, 9, and 10 in the Noon period, or on day 1, 2, 3, 7, 9, and 10 in the PM period. The maximum number of pairs performing this behavior was 3 (20%) on day 4 in the AM period.

Daily behaviors

Calves started to approach the water bucket from day 2 of life. On day 4 of life, 12 (80%) pairs placed their heads inside the bucket. All pairs placed their heads inside the water bucket from day 8 of life. Calf starter was provided from day 4 of life; on day 6 of life, a total of 7 (46.6%) pairs approached and placed the heads inside the bucket. From day 8 of life, 12 (80%) pairs placed their heads inside the calf starter bucket. Finally, all pairs required training or assistance to drink milk from the nursing bottles on days 1 and 2 of life. However, from day 5 to 10, only 2 (15%) pairs needed assistance.

Health, disease presentation, and performance

At least one calf from each pair (n=58, 100%) had diarrhea (fecal consistency 2 or 3) in the first 30 days of life. Forty-three (74.1%) pair mates were diagnosed with diarrhea within five days. Interestingly, out of those 43 calves, 25 (58.1%) were diagnosed with diarrhea on the same day, indicating concurrent exposure.

In relation to moderate diarrhea, 30 (51.72%) calves presented signs of moderate diarrhea, and 14 (24.1%) pair mates developed signs within 5 days (P=0.015). However, ten pairs (71.4%) were detected with moderate signs of clinical diarrhea on the same day.

Overall, 6 calves died due to diarrhea, and one calf was sold to another facility during the first month of life. None of those calves was from the same pair. The mortality for the observed period was 5.17%, and the mean age for death was 16.3±1.7 days of life. Finally, the mean (±SE) weight at day 30 of life was 52.7±0.53 kg, and the mean ADG (±SE) was 451.9±15.1 g/day for the study period (Table 4.4).

Discussion

To the best of our knowledge, there is limited information about the changes of the daily behavior of pair housed calves in the first days of life. This period might represent an adaptation to the companion and housing. Therefore, it is of interest to know when calves start to interact with each other. Research has mainly focused on analyzing behavior in more extended periods by selecting specific time points throughout the pre-weaning life (Chua et al., 2002; De Paula Vieira et al., 2010; Jensen et al., 2015; Bolt et al., 2017; Liu et al., 2020).

Weather conditions

This study was performed during the summer months in Colorado. Temperature humidity index above 72 units had been widely used as a cutoff point for heat stress in dairy cattle (Armstrong, 1994). Additionally, De Rensis et al. (2015) identified that mild signs of heat stress could be appreciated in dairy cows with THI above 68 units. Even though there is not a threshold to define heat stress in calves, scientific evidence suggests that calves exhibit physiological changes, such as increased rectal temperature and respiratory rate, when THI is over 65 units

(Dado-Senn et al., 2020). Additionally, a national study described that calves exposed to THI ≥ 70 units had a reduced performance in comparison with calves exposed to lower THI (Shivley et al., 2018). This information is relevant for this study; enrolled calves were exposed to environmental stress that might have affected their behavior, health, and performance, as discussed previously in Chapter 3.

The greatest THI for the first 10 days in pair housing was observed in the Noon period. Armstrong (1994) described that THI between 80 to 90 units was considered as moderate heat stress (Armstrong, 1994). Furthermore, the AM period was the only observed time when calves were not exposed to heat stress. Research has demonstrated that providing shade, fans, or evaporative cooling reduces the detrimental consequences of heat stress in adult and young cattle (Hill et al., 2011; Dado Senn et al.,2020; Polski and von Keyserlingk, 2017). In this facility, all the back windows of the hutches were opened, and the hutches were lifted from the back with bricks to enhance ventilation. Despite the efforts, THI inside the hutch was not reduced to comfortable limits, and it was 2 to 4 units higher than the environmental THI.

Baseline measurements

Enrolled calves fulfilled the latest recommendations for the transfer of passive immunity in rearing facilities (Lombard et al., 2020). It is recommended that at least 40% of the calves have an Excellent passive immunity (STP ≥ 6.2 g/dL); in this study, 80% of the calves were in that category, and no calves were in the Poor category (STP < 5.1 g/dL). In relation to birth weight, an average of 43.2kg was reported for Holstein heifer calves (Shivley et al., 2018). Consequently, this group of calves was lighter than the national average. Although, birth weight equal or greater than 36 kg has been considered as normal birth weight for Holstein heifer calves (Garcia et al., 2021)

Behavioral time budget

From the video recording, calves spent between 75 to 107 minutes resting per period in the first 10 days of pair housing. This represents between 63 and 89% of the observed time, which agrees with the 17 hours per day (70%) of lying time reported by Wormsbecher et al. (2017) for individual and pair housed calves in the first week of life. In addition, it was reported that calves spent 70% of their time lying during the pre-weaned life, independent of the housing system (Chua et al., 2002). Knauer et al. (2020) affixed calves with an accelerometer sensor, the mean lying time was 19 hours (79%) per day for the first week of life in pair housing. The main difference in resting time was observed when comparing day 1 (PM) and day 2 (AM, PM) to the rest of the days in observation. It was reported that 4 days old calves spent close to 20 hours per day resting (Hänninen et al., 2008). Therefore, it was expected to observe calves spending more time resting or laying in the first days of life. Faerevik et al. (2007) found that 40 day old calves increased their lying time for the first 2 days after regrouping. The authors associated this finding with the stress related to a new environment and a new group of animals. Even though the calves in our study are not comparable in age to the regrouping study, our findings might be associated with the adaptation to the new environment and pair mate.

A similar pattern was observed for the active time; calves spent 11 to 37% of their time being active in the AM and PM periods. Jensen et al. (1998) described that 2 weeks old calves housed in pairs spent around 25% of the time active. Contrarily, Chua et al. (2002) reported that pre-weaned calves spent around 1% of their time moving in the first 7 weeks of life. This discrepancy might be due to differences in the definition of active and moving time. Furthermore, in the PM period was possible to appreciate a more stable time budget for active time starting at day 3. Finally, in the Noon period, calves spent the greatest amount of time resting and the least amount of time being active. Therefore, the most likely explanation is linked to the THI calves experienced at this period.

In relation to play behavior, the results were presented as the percentage of the active time that calves engage in play behavior. Therefore, it is important to acknowledge that active time varied by day and period observed. Consequently, percentages for each play behavior might not be comparable over time. Overall, calves spent more percentage of time performing any type of play behavior in the PM period, followed by the AM period. In these periods, calves were on average more than 50% of the active time engaging in play behavior. At noon calves spent less than 49% of the active time performing play behavior. A study reported that the mean duration of play behavior at 15 days of life was of 350s/24h for calves fed 9L of milk per day (Jensen et al., 2015). For our calves, a minimum of 4 minutes and a maximum 32 minutes of play behavior was recorded. Therefore, our calves spent considerably more time engaging in play behavior per day.

One of the main differences in play behavior was the reduction in the percentage of locomotor play from days 1 and 2 with respect to the rest of the days in observation for the AM and PM periods. Locomotor play was reduced from 37 to 18% in the AM and from 48 to 30% in the PM. A study assessing locomotor play in pair housed calves with different space allowances found that pre-weaned calves spent on average 0.5% of the active time performing locomotor play. However, when considering only 2 week old calves, the time budget for locomotor play was less than 0.4% of the active time (Jensen et al., 1998). In the case of Jersey heifer calves housed in pairs, it has been described a locomotor play of 0.66% per hour (Pempek et al., 2016). Calves in these studies were offered 4.6L and 5 L of milk per day. Milk allowance is a critical factor associated with play behavior in calves; hunger may limit the likelihood for movement or play (Krachun et al., 2010). Another study described that 15 day old calves spent on average 231s/24h performing locomotor play (Jensen et al., 2015). When converting our percentages to minutes, the calves in this study spent a minimum of 2 minutes per period performing locomotor play, which is still a greater amount of time when considering the complete day in observation by the summation of the 3 periods.

Moreover, locomotor synchronized play behavior and social play behavior increased over time. Interestingly, a greater percentage of social play behavior was observed from days 2 to 7 in the AM period. It has been described for 12 day old calves a duration of active social time of 3.6 minutes per 3 hours of observation (Duve and Jensen, 2012). However, the definition of social time was different from our definition of social play behavior. In Jersey calves, social play in pair housed animals had been described as the 0.06% per hour around feeding time (Pempek et al., 2016). Finally, social play for 15 day old calves has been described as 69 seconds/24 hour (Jensen et al., 2015). The minimum mean time our calves spent in social play was 1.9 minutes in the Noon period. Therefore, the calves in this study spent in average more time engaging in social play behavior than in published studies. It is critical to remark that the variation in play behavior was important between pairs of calves and days in observation. Therefore, these results should be taken with caution.

Additionally, in the AM period, calves reduced their time alone in 40 minutes by increasing their time together inside the hutch in 30 minutes, and outside the hutch in 13 minutes over the days observed. Whereas, in the PM period calves increased their time together outside in 24 minutes. However, for the complete observational period, calves spent the least amount of time together outside the hutch. Contrary to our observation, it was found that pair housed calves spent 40.6% of the time inside the hutch and 58.7% of the time outside the hutch during the summer months (Wormsbecher et al., 2017). However, the difference in the time spent outside or inside the hutch was taken as the mean for the first 7 weeks of life of calves. It was concluded that an increase in the percentage of time spent together outside was for a restriction of space inside the hutch as the calves grew and improved thermoregulation in a combination of the warm temperatures (Wormsbecher et al., 2017). Therefore, the age of the calves made difficult to compare our results to the published study.

In the Noon period, the amount of time that calves spent together outside was very similar during the days in observation. Interestingly, calves spent considerably more time alone or together inside the hutch rather than together outside the hutch. Even though the THI inside the hutch was between 79 to 81 units, calves were not provided shade for the setting of the housing. It has been described that lactating cows spent more time in shade on days with higher solar radiation levels (Schütz et al., 2010). Therefore, calves might have sought shade inside the hutch during this period.

Binary behaviors

There was no difference in the frequency that calves were found together or alone at the start of each period. Interestingly, in the AM period, pairs increased the number of times they were found together starting from day 2 of life. A potential explanation might be related to the lower THI calves experiences at night-time and early morning.

Agonistic behaviors, such as contacting, displacing, and cross-sucking, are not desirable behaviors that may occur in the presence of companion at feeding time (Wormsbecher et al., 2017). In this study, those behaviors were not performed daily or frequently. Probably due to management practices, such as the installation of the bottle holders in opposite corners, the use of a physical barrier, and the milk allowance. Wormsbecher et al. (2017) found that paired calves had 0.8 to 1.4 bouts of displacing/hr and 0.1 to 0.4 bouts of cross sucking/hr when fed 16 L per day and the milk was offered on opposite sides of the pen. In addition, when milk was provided ad-libitum, 0.05% and 0.15% of paired housed calves engaged in nipple displacement or cross-sucking, respectively (Chua et al., 2002). Moreover, calves fed 10L of milk were observed cross-sucking only 5 times in 14 weeks of observation (Whalin et al., 2018). An elimination of displacing at feeding time was described when calves were provided a physical barrier of 100cm (Jensen et al., 2008). However, the barrier did not affect the presentation of cross-sucking, and over 44% of the calves performed cross-sucking at week 1 and 2 of life; calves were offered 6 L of milk per day (Jensen et al., 2008). In our study, an opposite situation happened; contacting was the behavior performed in a greater percentage and cross sucking the least frequent.

An important factor affecting this study is the number of pairs observed. Therefore, one or few pairs represent a relatively significant percentage. From our study and the literature cited, we can conclude that different management strategies can reduce agonistic behaviors at feeding time; nonetheless, these behaviors are not eliminated.

Daily behaviors

This group of 15 pairs of calves was approaching and placing their heads inside the water bucket in a high percentage since day 4 of life. By the end of the 10 days in observation, all pairs were approaching at least in one of the time periods observed. Supporting our finding, it was reported that calves that were offered water since birth consume 0.75 ± 0.05 kg/d water in the first 16 days of life (Wickramasinghe et al., 2019). Potentially, an important factor stimulating the water consumption was related to the temperatures observed throughout the study period. Even though the synchrony in this behavior was not assessed, by observation of the video recording, calves imitated the behavior of the pair mate placing the head inside the water bucket. Social learning or social facilitation are concepts used to describe this type of behavior; when by observation or interaction with another individual, there is an increase in the likelihood of the other calf to perform the same behavior (Costa et al., 2015). From the video recording, it was not possible to assess the consumption of calf starter. However, calves started to approach and place their heads inside the starter bucket from day 6 of life. More often, calves placed the head in the starter bucket after consuming water.

In addition, most pairs only needed training at the feeding time in the first 2 days in the housing. Although, 2 pairs of calves required assistance throughout the 10 days in observation. Therefore, it is critical to actively monitor the milk feedings of young calves in social housing. It is essential to assess that all calves have access to the offered milk and intervene when calves need assistance to avoid agonistic behaviors or hunger.

Health and performance

Disease transmission has been one of the main constraints for housing pre-weaned calves in pairs or groups (Costa et al., 2016). In this group of calves, the high incidence of diarrhea was unexpected, and it was discussed in depth in Chapter 3.

From our results following 58 pairs of calves, there was a high incidence of diarrhea within pair, where 74% of the pairs were detected sick within 5 days. However, 26% of the pair mates did not develop signs of diarrhea, independent of the close contact with the other calf and their feces. In terms of the severity of the diarrhea event, only 24% of the pairs had moderate or severe signs of disease within 5 days. Most of the calves that developed clinical disease were detected on the same day.

To the best of our knowledge, there are no published studies describing the disease presentation within pairs. In a study that compared different housing systems and ages at pairing, 98% of enrolled calves presented fecal scores greater than zero. The authors conclude that abnormal fecal scores are a common finding in young calves independent of the housing system (Jensen and Larsen, 2014). Pempek et al. (2016) described a low prevalence of diarrhea and other signs of clinical disease in a study following calves housed individually or in pairs. However, calves were evaluated for health outcomes only once a week (Pempek et al., 2016). Another study found a frequency of diarrhea that was approximately 5% for the first week of life (Liu et al., 2020). When comparing our results, it is essential to consider that this study was performed in a commercial facility under organic certification. Additionally, the study was conducted in a challenging environment due to the climatic conditions.

In relation to mortality, 5% of the calves enrolled died due to diarrhea in the first 30 days of life. In the US, the mortality described for the entire pre-weaned period is 5% (Urie et al., 2018b), and the recommendation of the Dairy Calf and Heifer Association is less than 3% (DCHA, 2016). Therefore, the percentage found in this study is high. Not surprisingly, the only cause of death was diarrhea, which has been described as the leading cause of mortality in pre-weaned calves (Urie et al., 2018b). It is important to mention, calves that died due to diarrhea were not from the same pair, and the mean age for diarrhea deaths is in accordance with the national average of 18.3 days of life (Urie et al., 2018b).

Additionally, the ADG in the first 30 days of life was 452 g/day for the 109 calve that completed the observational period. It has been described that excellent ADG for the pre-weaned period is greater than 820 g/day (Shivley et al., 2018). Although in this study, calves were followed only for the first 30 days, the mean ADG is poor (<640g/day) in comparison to the recommendations (Shivley et al., 2018).

Lastly, it is necessary to mention that our health assessments were performed only three times a week and not daily. For that reason, some differences in the detection of disease might have occurred. Despite the high incidence of disease at this facility, it is encouraging that pair housing does not necessarily imply that both calves will develop digestive disease, the same severity status, or die. For prevention and management practices, it is important to remark that majority of the pairs that developed diarrhea were detected on the same day.

Conclusion

The behavior of dairy calves, based on evaluation of 15 calf pairs, changed in the first 10 days of pair housing. Overall, heifer calves reduced their resting time after the first 2 days of life, and slowly increase their active time, synchronized and social play behavior, mainly in the morning and night feedings. Calves reduced their time alone by increasing their time together, especially inside the hutch. The noon feeding was characterized by elevated THI, which influenced the behavior of calves, making them rest more and spend more time alone or inside the hutch. Agonistic behaviors were not eliminated, although they were observed in low frequency. Concerning health in the first 30 days of pair housing, not all calves within a pair presented diarrhea or moderate to severe signs of disease. Additionally, calves that died were not from the same pair, despite the high incidence of diarrhea.

Tables and Figures

Table 4.1: Behaviors observed in the first 10 days of pair housing. Adapted from Jensen et al. (2008), Duve and Jensen (2012), and Pempek et al. (2016)

Behavior	Variable	Unit	Description
Together	binary	Yes/No	Calves together outside or inside at the beginning of period
Time resting	continuous	min/120min	Calf laying down
Time active	continuous	min/120min	Total time in observation minus resting time
Locomotor play	continuous	%[a]	Calf alone: gallop, leap, jump, head/body shake, bucking, running
Synchronized play	continuous	%[a]	Same as locomotor play but synchronized
Social play	continuous	%[a]	Butting, rubbing heads, neck. Front to front
Time as pair inside hutch	continuous	min/120min	Time together as pair inside hutch
Time as pair outside hutch	continuous	min/120min	Time together as pair outside hutch
Time alone	continuous	min/120min	Time alone inside or outside hutch
Contacting	binary	Yes/No	Calf is physically pushing the pair mate to remove it from the teat
Displacing	binary	Yes/No	Calf causing the pair mate to leave the teat at feeding time
Cross sucking	binary	Yes/No	Calf is sucking on the head, skin of the neck or under the belly of the other calf
Water intake[b]	binary	Yes/No	Calf spending time in the water bucket. Head inside bucket
Starter intake[b]	binary	Yes/No	Calf spending time in the starter bucket. Head inside bucket
Training/assistance at milk feeding	binary	Yes/No	Worker entering the calf unit to help calves to drink from the nursing bottle

[a] Percentage of the active time

[b] Intake was observed in video recording, it was not measured.

Table 4.2: Scoring system used to assess health status in the enrolled calves (modified from Wattiaux, 2005; McGuirk, 2008b; Smith 2009; and Perino and Apley, 1995)

Health variable	Score 0	1	2	3
Diarrhea	Normal	Semi-formed, pasty	Loose, but stays on top of bedding	Watery, sifts through bedding
Ear Position	Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
Nasal discharge	Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
Ocular discharge	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
Cough	None	Spontaneous cough	-	-
Attitude	Normal, no signs of depression, vigorous suckling	Mild depression, no signs of weakness, actively follows movements, suckle but not vigorously	Moderate depression, slight altered gait, able to stand, moves slower than pen mate, weak suckle	Severe depression with incoordination, altered gait, unable to stand or suckle
Dehydration	Normal, eyes are bright, no eyeball recession, skin tent <1s	Mild, eyeball recession 2-4mm, skin tent <3s	Moderate, eyeball recession 4-5mm, skin tent 3-5s	Severe, eyeball recession >6mm, skin tent >5

Table 4.3: Scoring system used to assess severity status in the enrolled calves.

Severity	Score	Description
Healthy	0	All health scores in 0
Mild	0	1 or 2 health scores in 1. Fecal consistency 2 with other scores in 0 or one health score in 1.
Moderate	1	Calves with 2 or more scores in 2. Fecal consistency 3 with 2 or more scores in 1 or one health score in 2
Severe	1	Calves with 2 or more health scores in 3. Calves with a health score in 3 and at least one health score = 2

Table 4.4: Descriptive statistics for baseline measurements, performance, and deaths for the first 30 days of life.

Variable	Mean	SE	Range
STP[a] (g/dl)	6.75	0.06	5.2-9.2
Birth weight (kg)	39.2	0.18	32.3-44.1
Weight 30 days (kg)	52.7	0.53	43.0-70.7
ADG 30 days (kg/d)	0.45	0.15	163.3-885.0
Death (days)	16.3	1.70	13-18

[a]Serum total protein

Table 4.5: Number and percentage of calves performing binary behaviors for the 10 days in observation by period.

Variable	No, n(%)	Yes, n(%)	Total, n(%)	P-value
Together				
AM	61 (49.6)	62 (50.4)	123(100)	0.25
Noon	67 (57.3)	50 (42.7)	117(100)	0.37
PM	71 (59.2)	49(40.8)	120(100)	0.71
Contacting				
AM	93(73.8)	33(26.2)	126(100)	<0.0001
Noon	103(81.8)	23(18.2)	126(100)	<0.0001
PM	90(75.0)	30(25.0)	120(100)	<0.0001
Displacing				
AM	113(89.7)	13(10.3)	126(100)	<0.0001
Noon	119(94.4)	7(5.6)	126(100)	<0.0001
PM	106(88.3)	14(11.7)	120(100)	<0.0001
Cross Sucking				
AM	116(92.1)	10(7.9)	126(100)	<0.0001
Noon	122(96.8)	4(3.2)	126(100)	<0.0001
PM	115(95.8)	5(4.2)	120(100)	<0.0001

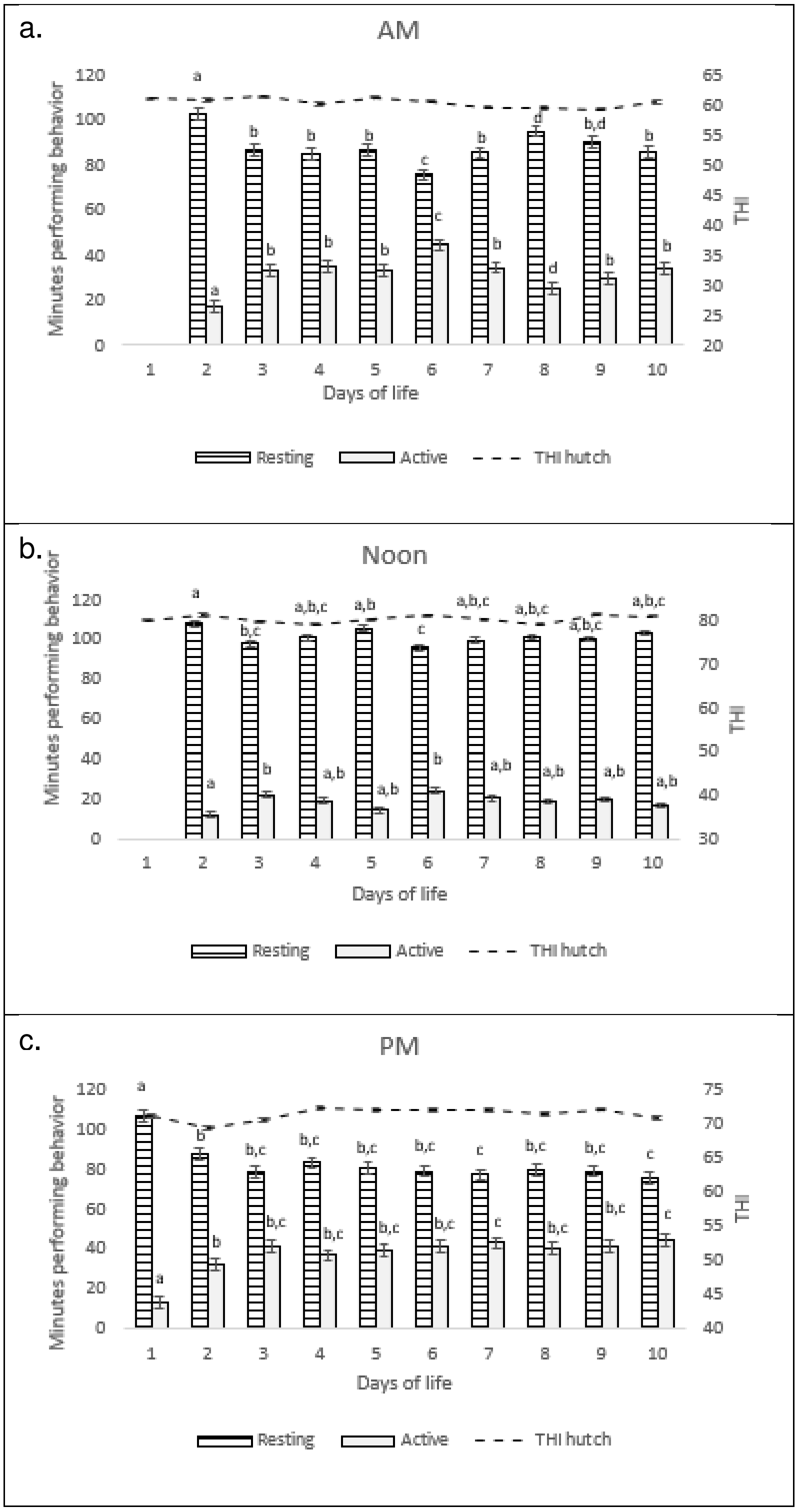

Figure 4.1: LSM for resting, active time, and THI in the first 10 days of life of calves housed in pairs (n=15) by period in observation. a) AM period (5:30AM to 7:30AM; $P_{resting}$<0.0001; P_{active}<0.0001). b) Noon period (11:30AM to 1:30PM; $P_{resting}$=0.22 ; P_{active}=0.22). c) PM period (6:30PM to 8:30PM; $P_{resting}$<0.0001; P_{active}<0.0001). Different letters indicate significant differences by performed behavior within periods. Resting time (horizontal lines), active time (solid color), THI (dashed line).

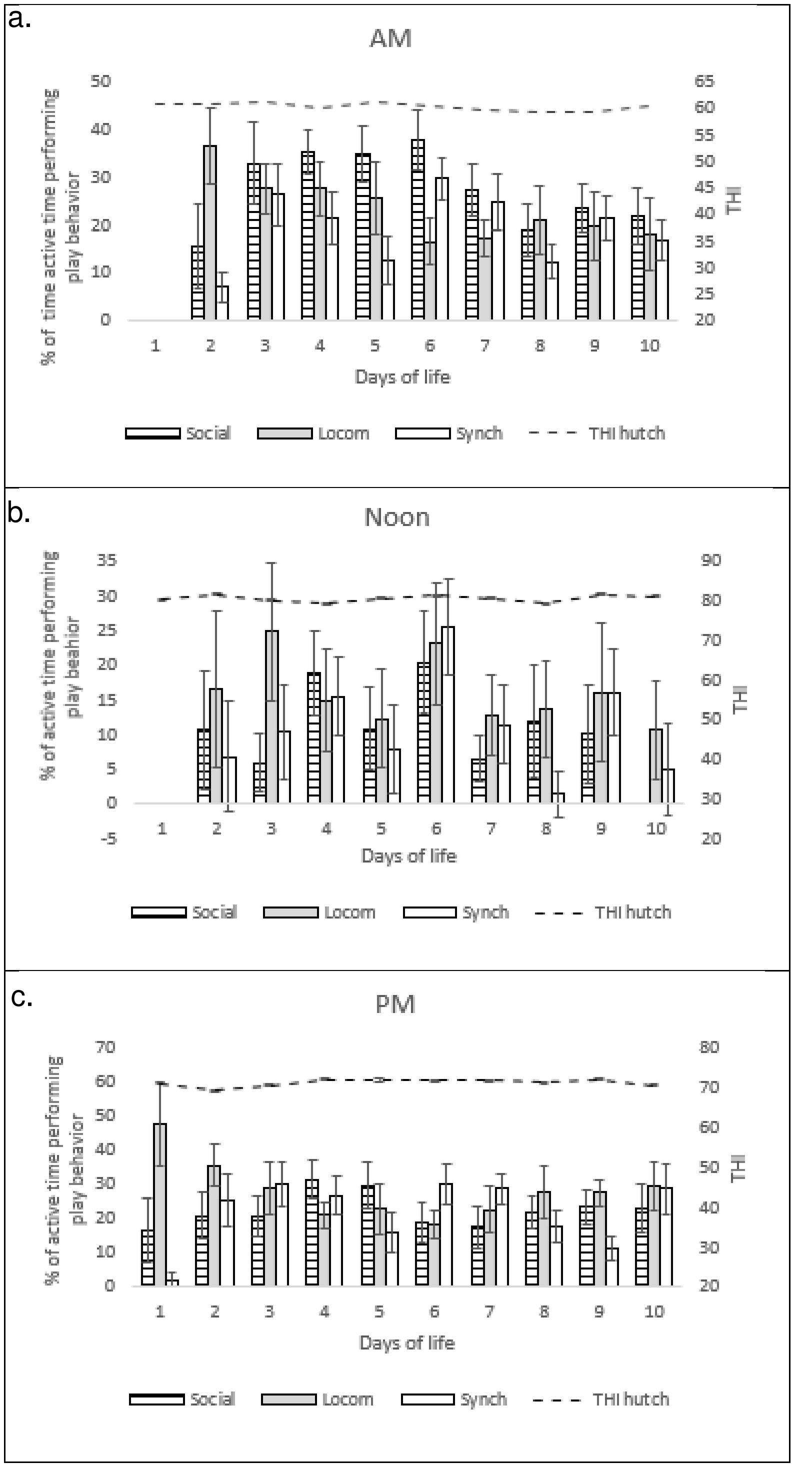

Figure 4.2: Percentage of the active time that calves (n=15) spent performing play behavior and mean THI in the first 10 days of life by period in observation. a) AM period (5:30AM to 7:30AM), b) Noon period (11:30AM to 1:30PM), and c) PM period (6:30PM to 8:30 PM). Social play (horizontal lines), locomotor play (solid grey), locomotor synchronized play (solid white), and THI (dashed line).

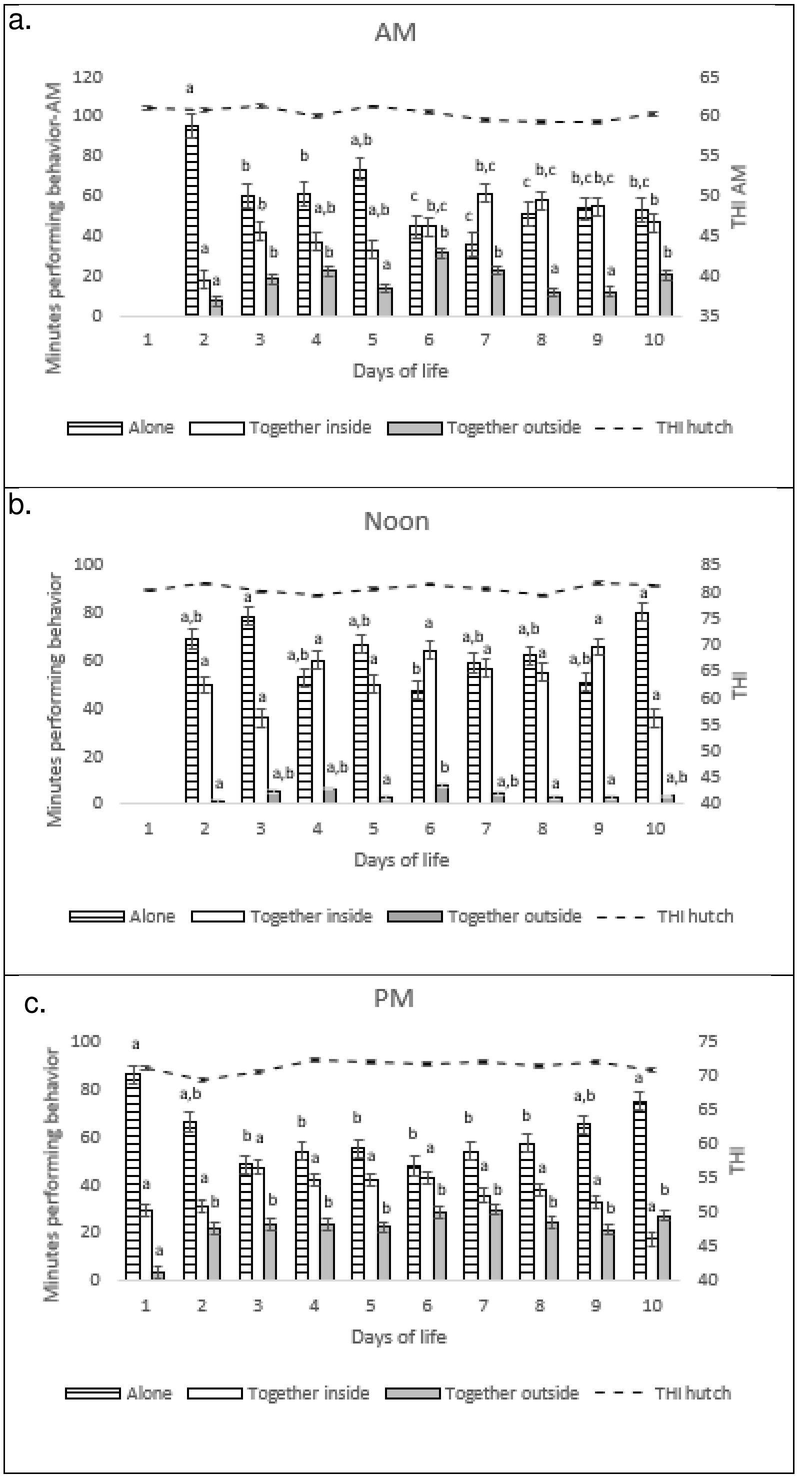

Figure 4.3: LSM for time alone, time together inside the hutch, time together outside the hutch, and THI in the first 10 days of life of calves housed in pairs (n=15) by period in observation. a) AM period (5:30AM to 7:30AM; $P_{alone}<0.0001$; $P_{inside}= 0.003$; $P_{outside}<0.0001$). b) Noon period (11:30AM to 1:30PM; $P_{alone}=0.31$; $P_{inside}=0.41$; $P_{outside}=0.21$). c) PM period (6:30PM to 8:30PM; $P_{alone}=0.6$; $P_{inside}=0.51$; $P_{outside}=0.004$). Different letters indicate significant differences by performed behavior within time periods. Time alone (horizontal lines), together inside the hutch (solid white), together outside the hutch (solid grey), and THI (dashed line)

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A2.1 Distribution of cows (n=192) that reached first or second or were culled before the first and second lactation (Lact 1, Lact 2) by serum IgG category (n=192), pre-weaned ADG (n=166), and pre-weaned disease events (n=192).

	Pre-weaned life predictor											
	IgG category (n,%)					ADG category (n,%)				Disease event (n,%)		
Variable	Excellent	Good	Fair	Poor	Total	Excellent	Fair	Poor	Total	Yes	No	Total
Lact 1												
Yes	65(34)	42(22)	25(13)	14(7)	146(76)	59(36)	43(26)	35(21)	137(83)	38(20)	108(56)	146(76)
No	13(6)	15(8)	11(6)	7(4)	46(24)	12(7)	8(5)	9(5)	29(17)	25(13)	21(11)	46(24)
Lact 2												
Yes	52(27)	29(15)	16(8)	11(6)	108(56)	42(25)	30(19)	27(16)	99(60)	28(15)	80(41)	108(56)
No	26(14)	28(15)	20(10)	10(5)	84(44)	29(17)	21(13)	17(10)	67(40)	35(18)	49(26)	84(44)

A3.1. Daily environmental and hutch temperature humidity index (THI) for the study period (June 21st to October 16th, 2020). Max: maximum THI of the day; Min: minimum THI of the day; Mean: daily mean THI; Mean day: mean THI for day hours (8AM-7PM); Mean night: mean THI for night hours (8PM-7AM).

	Environmental THI					THI Inside Hutch				
Date	Min	Max	Mean day	Mean day hours	Mean night hours	Min	Max	Mean day	Mean day hours	Mean night hours
7/21/2020	58.5	77.9	68.3	73.2	63.4	56.9	83.6	69.9	77.3	62.5
7/22/2020	57.2	77.8	69.2	74.2	64.3	55.4	81.7	69.9	77.1	62.8
7/23/2020	64.1	78.7	71.8	75.9	67.7	63.1	82.4	72.7	78.8	66.5
7/24/2020	64.6	77.8	70.0	73.5	66.5	63.9	83.6	70.6	75.4	65.8
7/25/2020	61.6	76.7	69.7	73.9	65.5	60.0	80.2	69.7	75.2	64.3
7/26/2020	63.5	72.8	68.5	70.9	66.2	60.0	76.7	68.5	72.8	64.3
7/27/2020	59.8	76.2	69.0	73.6	64.5	57.7	81.0	70.2	77.6	62.9
7/28/2020	58.9	76.6	67.9	72.7	63.2	56.7	80.0	68.3	75.6	61.0
7/29/2020	54.5	76.4	66.9	73.1	60.6	52.2	82.0	67.5	76.0	59.0
7/30/2020	56.9	70.6	64.4	68.2	60.6	54.7	73.2	64.3	69.9	58.7
7/31/2020	52.6	75.9	66.1	72.8	59.4	50.2	81.0	67.0	76.4	57.5
8/1/2020	55.5	76.0	66.6	72.0	61.3	52.8	81.6	67.1	75.2	59.0
8/2/2020	57.8	75.4	67.7	71.5	63.8	56.5	79.9	68.4	74.8	62.0
8/3/2020	57.3	75.4	68.0	72.2	63.7	56.0	79.6	68.9	75.2	62.5
8/4/2020	58.7	77.9	68.4	73.0	63.9	57.4	82.3	68.3	74.7	62.0
8/5/2020	59.6	76.3	67.8	72.4	63.2	57.6	80.2	67.9	74.6	61.1
8/6/2020	56.2	78.8	68.1	73.7	62.5	54.6	83.5	68.7	76.7	60.8
8/7/2020	55.5	77.7	68.3	74.8	61.9	53.4	82.1	68.8	78.3	59.2
8/8/2020	61.4	77.7	69.9	74.8	65.0	57.2	81.6	69.6	77.3	61.9
8/9/2020	53.8	77.6	69.0	75.0	62.9	50.9	81.9	69.4	78.2	60.5
8/10/2020	59.4	75.1	68.3	71.7	65.0	58.8	80.0	69.4	75.3	63.4

Continuation A3.1

8/11/2020	56.9	78.4	69.0	73.7	64.3	55.1	83.5	69.7	76.8	62.6
8/12/2020	62.3	76.9	69.9	73.6	66.2	61.1	81.6	70.4	76.1	64.7
8/13/2020	58.8	77.8	70.0	74.5	65.6	55.7	82.3	70.6	77.8	63.5
8/14/2020	58.8	74.8	67.4	71.7	63.2	55.1	78.1	66.5	73.1	59.9
8/15/2020	56.7	78.2	68.9	74.3	63.6	54.3	81.9	69.1	77.1	61.2
8/16/2020	58.9	78.6	70.5	76.2	64.7	56.8	83.6	71.1	79.4	62.8
8/17/2020	60.5	79.7	71.7	76.3	67.0	58.2	83.9	72.4	80.0	64.9
8/18/2020	58.6	79.3	70.2	76.4	64.0	55.4	84.2	70.6	79.3	61.8
8/19/2020	61.0	78.0	70.5	75.0	65.9	59.6	82.9	71.6	78.3	64.9
8/20/2020	57.5	77.5	69.1	74.7	63.4	55.7	80.1	69.0	76.3	61.6
8/21/2020	58.5	77.7	69.5	74.8	64.3	56.2	81.2	69.9	77.4	62.4
8/22/2020	57.3	79.2	69.1	75.4	62.8	54.0	82.4	68.9	77.5	60.2
8/23/2020	57.3	79.0	69.3	74.9	63.7	55.7	82.5	69.2	77.4	60.9
8/24/2020	61.4	80.6	71.4	76.3	66.6	60.3	84.5	71.9	79.1	64.8
8/25/2020	62.2	81.2	71.6	76.3	66.9	60.3	85.9	72.2	79.0	65.5
8/26/2020	65.6	79.3	71.6	75.9	67.4	64.8	80.4	71.1	76.1	66.2
8/27/2020	57.8	78.5	68.9	74.9	63.0	55.3	82.7	69.3	77.9	60.8
8/28/2020	61.6	72.5	66.8	69.6	64.0	59.3	74.0	66.5	70.2	62.7
8/29/2020	55.1	71.4	63.3	67.8	58.7	52.8	73.6	63.4	70.1	56.7
8/30/2020	53.4	76.0	65.9	73.3	58.4	50.6	80.7	66.0	75.5	56.4
8/31/2020	49.2	69.0	59.5	64.4	54.6	45.8	70.8	60.0	66.8	53.2
9/1/2020	48.4	71.6	61.2	67.2	55.2	45.4	76.0	62.0	70.8	53.1
9/2/2020	52.3	75.4	64.1	70.9	57.4	48.4	78.1	64.0	73.8	54.2
9/3/2020	53.5	71.1	64.2	67.6	60.7	50.4	74.4	65.0	70.7	59.2
9/4/2020	49.0	75.2	64.6	72.1	57.1	47.6	79.1	64.4	74.4	54.5
9/5/2020	54.2	79.6	66.9	74.4	59.3	50.4	82.7	66.5	76.1	56.8
9/6/2020	58.2	78.3	69.2	75.5	62.8	55.3	81.4	68.2	76.3	60.1
9/7/2020	45.8	70.3	60.5	65.1	55.9	46.0	75.0	60.7	66.2	55.2

Continuation A3.1

9/8/2020	32.5	43.1	36.0	35.6	36.4	33.5	43.2	36.2	35.7	36.7
9/9/2020	32.3	45.3	37.4	40.4	34.5	32.9	49.2	38.0	42.2	33.8
9/10/2020	36.2	55.0	46.6	51.3	41.8	35.3	61.7	47.9	55.0	40.9
9/11/2020	41.9	60.2	51.1	55.0	47.2	42.3	66.6	52.8	59.0	46.6
9/12/2020	40.5	72.0	57.5	66.6	48.5	38.5	74.2	56.8	67.8	45.8
9/13/2020	44.5	72.6	60.7	68.6	52.9	40.7	76.3	60.1	70.4	49.7
9/14/2020	44.5	74.2	61.3	68.4	54.1	42.8	76.6	60.7	70.4	51.0
9/15/2020	48.3	74.1	61.9	69.8	54.0	45.8	77.3	61.4	71.4	51.3
9/16/2020	54.5	68.9	62.1	65.7	58.5	50.2	73.2	61.6	67.5	55.7
9/17/2020	44.9	74.3	59.4	67.5	51.3	42.1	77.4	59.3	69.3	49.3
9/18/2020	48.1	72.4	60.5	66.2	54.9	45.2	75.1	60.4	68.6	52.2
9/19/2020	52.7	75.1	64.4	70.8	58.1	50.0	78.2	64.3	72.4	56.3
9/20/2020	55.8	73.8	65.4	69.1	61.7	52.7	76.8	65.5	70.5	60.5
9/21/2020	50.8	74.3	63.6	70.1	57.1	47.4	76.9	63.4	71.8	55.0
9/22/2020	51.2	74.9	64.3	70.6	58.0	47.4	78.0	63.8	72.2	55.3
9/23/2020	54.4	73.8	65.4	70.2	60.7	52.6	76.7	64.4	71.0	57.8
9/24/2020	51.3	75.1	63.4	69.8	57.1	47.7	76.8	62.7	70.9	54.4
9/25/2020	51.1	73.6	62.3	68.1	56.5	48.4	76.7	61.8	69.9	53.7
9/26/2020	56.0	72.2	63.0	68.0	58.1	52.3	74.6	62.5	69.1	55.9
9/27/2020	43.0	56.0	51.6	52.5	50.8	39.5	56.7	50.8	52.7	48.9
9/28/2020	37.5	61.8	50.7	57.2	44.3	32.9	64.5	49.4	59.1	39.8
9/29/2020	39.1	71.2	56.2	64.9	47.4	36.7	74.4	54.9	66.3	43.4
9/30/2020	49.9	66.7	58.4	63.8	53.0	45.6	70.4	57.2	64.9	49.5
10/1/2020	38.6	63.7	52.7	58.6	46.8	35.9	68.6	52.1	61.0	43.3
10/2/2020	42.0	69.0	56.1	64.5	47.8	38.3	71.6	55.2	65.1	45.4
10/3/2020	46.6	65.0	54.8	60.1	49.4	44.1	69.2	54.2	62.6	45.9
10/4/2020	39.7	70.6	55.7	63.8	47.5	36.9	72.5	54.5	65.1	43.9
10/5/2020	49.0	70.8	59.0	65.7	52.2	45.3	73.0	57.6	66.4	48.7

Continuation A3.1

10/6/2020	40.8	72.1	56.5	65.2	47.7	36.8	74.0	55.0	66.0	44.1
10/7/2020	44.3	73.2	59.5	67.3	51.6	40.8	76.9	58.1	68.6	47.7
10/8/2020	44.1	72.2	58.0	64.3	51.7	39.9	75.0	57.1	65.7	48.5
10/9/2020	49.4	72.1	60.6	66.8	54.3	46.3	74.6	59.2	67.5	50.8
10/10/2020	47.7	72.1	59.2	65.1	53.3	45.9	73.5	58.1	65.6	50.7
10/11/2020	49.0	67.2	55.9	59.1	52.7	44.7	69.2	54.9	60.1	49.8
10/12/2020	38.9	66.5	54.8	60.5	49.0	33.8	68.7	53.3	61.0	45.7
10/13/2020	46.8	68.3	56.3	60.9	51.8	42.6	69.9	55.8	62.6	48.9
10/14/2020	43.8	63.3	52.9	56.4	49.4	42.5	63.2	51.5	56.2	46.9
10/15/2020	35.2	55.0	44.0	48.6	39.4	32.7	60.5	44.5	51.0	37.9
10/16/2020	26.0	59.4	44.2	51.8	36.6	22.3	66.6	48.1	57.3	38.9