IMPACT OF FEEDING MANAGEMENT ON GROWTH, METABOLISM, AND HEALTH OF PREWEANED DAIRY CALVES DURING SUMMER IN THE SOUTHEASTERN REGION OF THE UNITED STATES

by

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(Under the Direction of Sha Tao)

ABSTRACT

Feeding more milk replacer (MR) in the summer may improve calf growth by increasing energy availability for maintenance and growth. Therefore, the objective of the first experiment was to evaluate the effect of different MR feeding programs on calf performance and metabolism during summer. Feeding 0.66 kg/d dry matter (DM) of a MR containing 26% crude protein and 17% fat (26:17) improved calf growth compared with feeding 0.55 kg/d DM of a MR containing 20% crude protein and 20% fat (20:20), but feeding 0.77 kg/d DM did not support further improvements in calf performance. Feeding MR more frequently when large amounts of MR are fed may improve energy utilization and alleviate heat stress. Consequently, the objective of the second experiment was to evaluate the effect of MR feeding rate (FR) and frequency (FF) on performance, health, abomasal emptying, nutrient digestibility and glucose metabolism during the summer and winter. Increasing feeding frequency in the summer, lowered respiration rate and rectal temperature, however no effect was detected on average daily gain (ADG), and nutrient digestibility. Feeding more frequently accelerated abomasal emptying and feeding more MR delayed abomasum emptying in the summer only. Increasing feeding frequency improved insulin action at the peripheral tissue level.

INDEX WORDS: Summer, Dairy calves, Milk replacer, Feeding rate, Feeding frequency, Growth performance

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DEDICATION

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CHAPTER 1

INTRODUCTION

Dairy calves are the future milk producers in a dairy farm. Growth and health in the calf's early life have a profound effect on her future performance. Raising healthy replacement heifers is very important to maintain a profitable rotation of animals in the dairy operation. Appropriate management and nutritional practices should be considered during gestation, parturition, preweaning, and post weaning periods to secure the following generation of lactating cows. Furthermore, growing replacement heifers represent the second largest expense on the dairy operation (Heinrichs, 1993). Therefore, calf survival is not only important from the welfare point of view, but also, economically efficient.

Environmental condition is a major factor affecting animal development, growth, and wellbeing. Climate in the southeastern region of the US is diverse, however states like Florida and Georgia have the warmest temperatures accompanied by elevated relative humidity. Undeniably, summer heat stress conditions in the Southeast is challenging for dairy cattle, and results in significant economic losses to the dairy industry worldwide (Key et al., 2014). However, although the negative impact of heat stress on lactating dairy cows is well recognized, the effects of heat stress on dairy calf are often overlooked. It is frequently believed that heat stress impacts neonatal calves to a lesser extent due to their low metabolic heat production per unit of surface area. However, calves exposed to high environmental temperatures that surpasses 20 °C have increased body temperatures and initiate evaporative cooling to dissipate heat accumulated during the day (Gebremedhin et al., 1981). Furthermore, calves reared during summer have lower preweaning average daily gain (ADG) (Wiedmeier et al., 2005) and higher mortality rates (Stull et al., 2008) compared with those raised during temperate environments, suggesting potential negative impacts of heat stress on calf growth and health. Similarly, preweaned calves raised under summer condition had lower growth rate relative to those under thermal neutrality (Chavez, 2011). The negative impact of heat stress on calf growth can be explained by lower energy supply for growth because of the increased energy requirement for maintenance (Gebremedhin et al., 1981) and reduced starter intake (Chavez, 2011). Therefore, finding management and nutritional strategies to reduce the negative impacts of heat stress on calves is of vital importance.

Implementing heat abetment to dairy calves has shown to improve performance and comfort. Shade installed above hutches in an open area reduces hutch air temperature, calf body temperature and respiration rate (Spain and Spiers, 1996). Furthermore, housing calves under shade immediately after birth improves passive immune transfer(Stott et al., 1976). Moreover, forced air ventilation by installing fans in the nursery barn increases calf ADG in the summer (Hill et al., 2011). However, nutritional strategies to improve performance in the summer have been scarcely explored. Hypothetically, increasing MR feeding rate could be an approach to increase energy and nutrient intake in the summer when calves demand more energy for maintenance and grain intake is reduced. Studies conducted in temperate environments in dairy and veal calves have shown that increasing MR feeding rate improved preweaning ADG (Diaz et al., 2001, Jasper and Weary, 2002). Increasing feeding rate in the summer could be a feasible alternative to improve growth, therefore research is needed to explore this hypothesis.

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CHAPTER 2

REVIEW OF LITERATURE

The importance of calf rearing

Growing heifers are the future lactating cows in a dairy farm, and their performance determines the future success of the farm. Rearing replacement heifers also represents the second largest cost for a dairy farm accounting for 20% of the overall operation cost (Heinrichs, 1993), requiring a significant financial investment (Gabler et al., 2000, Tozer and Heinrichs, 2001). However, a study carried out in 19 dairies in the United Kingdom estimated that only 85% of heifers born alive reached first lactation, and 19% left the herd before the second lactation (Brickell et al., 2009, Brickell and Wathes, 2011). This represented a significant economic loss to the dairy producers. Furthermore, Tozer and Heinrichs (2001) identified that the age at first calving was the most influential factor affecting the cost of rearing replacement heifers. This is because older heifers at first calving have higher feed cost. Additional heifers are also needed to meet replacement needs reducing the number of surplus heifers that could be sold. The investment to a dairy heifer will only be recovered when she begins to produce milk; therefore, good health and survival of the calf are paramount to improve farm profitability.

Early life events of a calf, such as stress, management, disease, and nutrition, not only influence calf performance before weaning, but can also have a profound effect on health, reproduction, and lactation performance during her future life (Britney et al., 1984, Heinrichs and Heinrichs, 2011). For instance, Jorgensen et al. (2017) surveyed 38 dairy farms that employed automatic calf feeders in Minnesota, northwest Iowa, and Wisconsin and concluded that factors associated with management such as milk or MR bacterial count, poor milk daily allowance and nutrient content significantly affect calf health. Heinrichs et al. (1987) surveyed 329 Pennsylvania dairies to gather information about dry cow management, calving areas and management, colostrum, housing, feeding managements, and health of calves and heifers. From this survey it was identified that feeding management related to the amount and type of feeds fed were the major concerns for famers and were highly correlated to preweaning calf mortality. It also has been demonstrated that successful immunoglobulins passive transfer at birth is associated with improved performance and health before calving and reduced age at first calving (Furman-Fratczak et al., 2011). Occurrence of diseases in early life had significant carry over effect on the calves' future performance. Correa et al. (1988) reported that calves with respiratory illness were two times more likely to calve 6 mon later than heifers without respiratory disease. Britney et al. (1984) found that calves with navel-joint infections within the first 4 mon of life had fewer days in the herd indicating a lower survival rate than the herdmates without infection. More recent studies reported that events occurring before birth, such as heat stress, could also affect health and metabolism of the preweaned calves and the milk yield of their first lactation (Guo et al., 2016, Monteiro et al., 2016a, , 2016b). Therefore, optimal management practices during the early life of the calve not only is essential for a successful rearing program but also ensure her productive lifetime.

Overview of calf management

Colostrum management

Early and adequate intake of good quality colostrum is an important factor determining calf health, pre- and postweaning survival, and future performance (Godden, 2008). A national survey conducted in 2014 (USDA, 2016) reported that the time of first colostrum feeding average 3.6 h after birth, and only 21.8% of operations fed 3.8 L or more of colostrum in the first feeding. Another survey of 113 dairy farms in the Midwest and Northeastern US reported that 61.9% of the farms provided 3.8 L of colostrum (Fulwider et al., 2008). These discrepancies represent enormous variations in colostrum management among dairies in the US. This variations partially explain the high heifer mortality rate (7.8 to 11%) observed in the US (USDA, 1996, 2016).

The NAHMS reported a 5% calf mortality rate (2,545 heifers considered, excluding calves that died before 24 h) of which 32% died from digestive problems and 7% from digestive and respiratory problems. In addition, diarrhea was the major cause for calf mortality during early life and most calves died within the first three weeks of age (USDA, 2016). In the same survey, calf morbidity was 33.8%, and half of the sick calves suffered from digestive problems. Additionally, most cases of diarrhea occurred during the first 2 wk of age and respiratory problems peaked at week 5 of age. In this survey, one major factor influencing mortality and morbidity was serum IgG concentration. Therefore, good colostrum management practices are important for calf survival during early life.

Colostrum quality, quantity, and timing at first feeding are important in a successful colostrum program. Colostrum quality is associated with IgG content. Desirable colostrum contains ≥ 50 g/L of IgG (Godden et al., 2019); however, factors such as breed (Guy et al., 1994), age of the cow (Shivley et al., 2018), maternal nutrition (Nowak et al., 2012), time at first milking (Moore et al., 2005) and dry period length (Grusenmeyer et al., 2006) affect colostrum quality. Therefore, colostrum quality should routinely be measured on farms. Time at first feeding and amount of colostrum fed are important. It is recommended that colostrum be fed at 10 to 12% of calf's body weight immediately after birth. Calves fed 4 L of colostrum at 0 h and 2

L more after 12 h had higher serum IgG concentrations at 24 h compared with calves fed 2 L of colostrum at 0 h and 2 L 12 h later (Morin et al., 1997). The importance of feeding colostrum within the first 2 h after birth rely on the rapidly gut closure leading to poor immunoglobin absorption (Fischer et al., 2018).

Pasteurizing colostrum at moderate temperatures (60° C) for 60 min have been to improve IgG absorption and lower the risk of feeding colostrum contaminated with bacteria. Pasteurization has minimal effect on the properties of the colostrum but eliminates pathogens (Godden et al., 2006, Donahue et al., 2012). A study that evaluated the effect of colostrum pasteurization on IgG absorption and health of 1071 neonatal calves from 6 farms in Minnesota and Wisconsin found that calves fed the heat treated, compared to fresh, colostrum had a higher serum IgG concentration at 18 to 15 mg/mL, respectively. Furthermore, feeding calves with unheated colostrum increased the risk of treatment for scour compared with those fed heated colostrum (Godden et al., 2012).

Housing

Calf housing should be dry, well-ventilated, accessible for calf management purposes, and easy to clean and sanitize (Davis and Drackley, 1998). Individually housing has been traditionally promoted to avoid disease transmission and improve performance of calves (Waltner-Toews et al., 1986, Quigley et al., 1994). According to the national surveys conducted in 2007 and 2014 (USDA, 2010, 2016), 74.9 and 86.6% heifers are housed individually, respectively. Individual calf hutches located either outdoors or under a cover with natural ventilation are the most common housing used for raising calves. On the other hand, one fourth of producers individually house calves in the indoor facility. Urie et al. (2018) utilized data from 2,545 heifer calves included in the USDA (2016) survey that included dairy farms located in 13 states in the West, Midwest and Northeast of the US. They reported that 13.4% of calves in 20.2% of participating dairy operations were housed in groups. Compared with individual housing, these researchers reported that group housing with ad libitum or accelerated milk or MR feeding program could improve feed intakes and weight gains, if proper management practices were implemented to reduce health risks (Costa et al., 2016).

Feeding management

Due to convenience, safety, consistency, and cost effectiveness, feeding MR to substitute or complement waste milk or whole milk in preweaning diets has been a common practice since the second half of 20th century (Davis and Drackley, 1998). Urie et al. (2018) reported that 34.8% of the calves were fed MR and 25.1% of calves were fed a mixture of milk and MR on 104 dairy operations in West, Midwest, and Northeast. These data indicated that MR was commonly used in diets for young calves in the U.S. Interestingly, Urie et al. (2018) also reported that calves enrolled in the survey consumed an average of 5.6 L liquid feed in 2.6 feedings per day.

Traditionally, the amount of milk or MR offered to young calves was limited in order to increase dry feed consumption. This practice accelerates rumen development and reduces the age at weaning. In contrast, increasing the feeding rate and protein content of the milk or MR has shown to improve calf current and future performance. The improvements included increased lean mass gain and feed efficiency, and reduced mortality rates before weaning, and enhanced post weaning health, younger age at first calving, and greater milk yield in the first lactation (Diaz et al., 2001, Jasper and Weary, 2002, Blome et al., 2003, Bartlett et al., 2006, Khan et al., 2007, Ollivett et al., 2012, Soberon et al., 2012). These findings have led to an increased use of the accelerated rearing programs that allow for feeding larger amounts of milk or MR with higher protein contents.

Although accelerated feeding programs have shown several short- and long-term advantages, there are concerns related to delayed weaning that could be counterproductive if managed incorrectly. Successful weaning process includes rumen development and metabolic adaptation. To accomplish these, the calf must begin consuming high fermentable carbohydrates early in her life (Warner et al., 1956, Sander et al., 1959, Tamate et al., 1962, Stobo et al., 1966, Baldwin et al., 2004). A recent study, compared a conventional MR feeding program [0.44 kg/d of a 21% CP, and 21% crude fat (21:21) MR on a DM basis] with the moderate and aggressive program [0.66 and 0.87 kg/d of a 27% CP and 17% fat (27:17) MR on a DM basis] on calf performance, intake and rumen development. Calves offered the conventional MR feeding program had the lowest ADG, feed efficiency, and structural growth compared with the other treatments. However, the aggressive treatment (0.87 kg/d of 27:17 MR) had the lowest preweaning starter intake and the lowest digestibility for OM and NDF postweaning (Chapman et al., 2016). Whether these may have negative effects on development of future performance deserves more research.

Increasing milk or MR allowance with limited feeding frequency also slows abomasum emptying (Burgstaller et al., 2017). Compared with the calves raised with their dam who "naturally" nurses 6-8 times/d, the calf enrolled in an accelerated feeding program normally consumes 8-10 liters of milk or MR in two feedings each day. This may lead to digestive issues such as abomasal bloating (Burgstaller et al., 2017; Geof Smith, North Carolina State University, personal communication). Therefore, feeding more than twice a day may be necessary when accelerated a feeding program is adopted. However, it is challenging for many modern dairies with more traditional labor systems. One of the major advantages of automatic calf feeders is to increase feeding frequency without increasing labor cost (Geof Smith, North Carolina State University, personal communication). Although automatic feeding systems are gaining in popularity, individually housing and feeding using nipple bottles or pales are still the dominant calf rearing practice in North America (USDA, 2016) (Medrano-Galarza et al., 2017). Therefore, identifying feasible solutions to minimize the potential health issues due to the increased MR feeding rate in conventional managed dairy operations is of vital importance.

The increased cost of feeding additional MR, extra labor, and increased age of weaning associated with accelerated feeding programs is a major economical concern to dairy producers (Heinrichs and Gelsinger, 2017, Hawkins et al., 2019). It is important to note that, in addition to the MR allowance, other management factors such as hygiene, mixing method, water temperature and at consistent feeding protocol all affect the successful adoption of the accelerate feeding program for dairy calves.

Cold stress

Cold stress is a significant issue for calves raised during winter and spring months. It is associated with impaired immunoglobulin absorption from colostrum (Olson et al., 1980), and contributes to the increase in calf mortality observed during the winter compared with other seasons (Martin et al., 1975). Furthermore, calves experiencing cold stress normally have increased grain intake to maintain body temperature (Gebremedhin et al., 1981). Compared with calves raised under thermoneutral conditions, calves in cold conditions consumed more grain but maintained similar BW during the preweaning period (Nonnecke et al., 2009). Deep bedding and insulated housing (Davis and Drackley, 1998, Nordlund, 2008), dry and warm calving area equipped with heat lamps (Butler et al., 2006), use of clean and dry calf jackets (Rawson et al., 1989), good colostrum management, and increasing energy intake (Davis and Drackley, 1998, Silva and Bittar, 2019) are crucial components to secure calf welfare, survival and performance during harsh winter conditions.

Challenges in the southeastern US

There are regional biases toward farms located in the Midwest, West, and northeast of the U.S to be enrolled in surveys of dairy management in this country. Far less information is available about dairy management practices utilized in the Southeastern United States. In general, management practices are similar for farms in all locations. These practices are based on a common set of recommendations (Mark Hill, Provimi North America, Inc., personal communication). The practices include, but not limited to, common colostrum feeding programs, liquid and dry feeding practices, calving handling management, and the choice of housing. One major difference between heifers raised in the southeast and in the rest of the US is the severity of the environmental conditions. While animals located in the northern states frequently suffer from cold stress, prolonged heat stress is only a major burden to cattle in the south, especially in the subtropical southeastern states (West, 2003)

Heat stress has a major negative effect on dairy industry worldwide. It impairs a cow's health, reproduction, production, and welfare, and causes significant economic losses for dairy producers. It has been estimated that the reduced milk yield caused by heat stress alone resulted in more than $1.2 billion annual losses in the dairy sector of the United States (Key et al., 2014). These negative impacts are amplified in the southeastern United States because of the high humid and prolonged high temperatures. Although the negative impacts of heat stress on lactating dairy cows is widely recognized, its effect on calves is often overlook. However, both controlled experiments and field studies suggest that calves raised under heat stress conditions have lower growth rates, increased disease incidence and higher mortality rates relative to those raised in the temperate environments or thermal neutral conditions (Stull et al., 2008, Broucek et al., 2009). These data suggest that heat stress is also a limiting factor for preweaned dairy calve growth and health.

The elevated relative humidity and environmental temperatures in the southeastern states exaggerate the negative impact of heat stress on dairy calves by preventing them from effective cooling (Mark Hill, Provimi North America, Inc., personal communication). For instance, animals raised in northern states or in arid environments, such as Arizona, experience significant nighttime cooling due to the lower relative humidity and reduced ambient temperature. This will effectively alleviate the heat load accumulated during the day. In contrast, calves raised in Georgia and Florida are exposed to persistent heat stress at night because temperatures and relative humidity remain elevated.

Thermoregulation

Maintaining body temperature within normal the physiological range is important for maintenance normal body functions (homeostasis). The body temperature of a cow is dependent on the balance between metabolic heat production and heat exchange of the cow with the environment. During heat stress the animal undergoes a series of physiological, neurological and metabolic changes to maintain normal body temperature by increasing heat loss and decreasing metabolic heat production (Collier et al., 2018). Heat can be exchanged by sensible heat transfer including convection, conduction, and radiation. These routes of heat exchange are dependent on the temperature difference between the cow's body surface and ambient environment. When ambient temperature exceeds the cow's surface temperature, sensible heat loss is ineffective and the animal relies on evaporative cooling to maintain body temperature (West, 2003). Different from sensible heat exchange, the effectiveness of evaporation (or latent heat exchange) is dependent on the relative humidity. As the relative humidity increases, evaporative cooling becomes less effective. Heat loss by evaporative cooling relies on the increases of respiratory and cutaneous heat loss. These are characterized by the increased respiration rate, sweating rate and cutaneous blood flow for heat dissipation of heat-stressed animals. Metabolic heat production is correlated with an animal's response to heat stress. Conversely, one major regulatory mechanism of the heat stress animal is to reduce metabolic heat production by limiting productivity and intake. While the upper critical temperature of mature cows is reported to be 25 to 26 °C, this is greatly dependent on the cow's previous adaptation to certain climates and lactation performance (Collier et al., 2017).

The direct impact of heat stress on the calf is often overlook compared with lactating dairy cows. This likely is because calves have lower heat production per unit of surface area, and theoretically calves are more efficient in to dissipating metabolic heat. However, when calves were challenged by high ambient temperature, they also have increase body temperature, perspiration, and respiration rate (Gebremedhin et al., 1981). Furthermore, calves initiate evaporative cooling through both cutaneous and respiratory mechanisms when environmental temperature exceeds 20 °C (Gebremedhin et al., 1981).These physiological responses suggest that calves also initiate heat dissipation under unfavorable heat stress conditions. Consequently, energy utilization for evaporative cooling will increase the energy demand for maintenance decreasing energy available for growth (Gebremedhin et al., 1981).

Prenatal heat stress and its effect on the offspring

The impact of heat stress on calf survival and performance begins during gestation. Maternal heat stress during the dry period has negative impacts on calf birth weight, body growth, survival, and future lactation performance (Monteiro et al., 2016a). Tao and Dahl (2013) reviewed the negative effect of heat stress during the late gestation on placental and mammary gland development. These are mediated through reduced vascularization and blood flow, and decreased tissue growth due to inhibited cell proliferation by heat stress. The impaired placental development limits the fetal uptake of oxygen and nutrients including glucose and amino acids. These directly limit fetal growth leading to lower birth weight (Tao and Dahl, 2013). Furthermore, Tao et al. (2012) found that calves born to heat-stressed cows who were not exposed to cooling had lower total serum IgG and apparent efficiency of IgG absorption and reduced peripheral blood mononuclear cells proliferation compared with calves born to cows maintained under active cooling during the dry period. These data suggested that late gestation heat stress impaired the calf's passive and cell-mediated immunity. Consequently, Monteiro et al. (2016a) reported that heifers born to non-cooled heat-stressed cows were more likely to leave the herd before the first lactation, had higher number of services per 1st pregnancy, and had lower milk yield during the first lactation than heifers born to cows under active cooling during the late gestation. Collectively, these data indicate that heat stress during late gestation have a profound negative impact on the offspring performance and that heat abetment during the dry period should be taken into consideration in the dairy operation.

Postnatal heat stress on intake and growth.

Similar to mature cows, calves experiencing high environmental temperatures in the summer have reduced starter intake compared with calves raised in cool seasons or under thermoneutral conditions (McKnight, 1978, Chester-Jones et al., 2008, Chavez, 2011). This, coupled with the increased energy expenditure to maintain body temperature, further limits the energy availability for growth. Bateman and Hill (2012) reported that as ambient temperature increased grain intake decreased, furthermore, for every 0.5°C increase in the body temperature of calves, there was a 15% reduction in ADG and structural growth. Similarly, Chavez (2011) observed lower body weight gain of calves exposed to heat stress conditions during summer compared with calves maintained in thermoneutral conditions, and partially attri buted this effect to the lower grain intake caused by heat stress. Consistently, calves raised in summer have lower ADG compared with those raised in cool seasons (Broucek et al., 2009).

In lactating dairy cows, 50% of the reduction in milk yield is explained by the decrease in DMI (Wheelock et al., 2010). Whether the reduction in feed intake is entirely responsible for the impaired growth of the dairy calves is still questionable. Compared with heat-stressed dairy bull calves, pair-fed thermoneutral animals had unchanged nutrient digestibility and similar reduction in ADG. These data suggest that the reduction in DMI could entirely explain the lower growth rate by heat stress in dairy calves (Yazdi et al., 2016). More research is needed to elucidate whether approaches that increase feed intake during summer would positively impact growth and health of preweaned dairy calves.

Postnatal heat stress on immunity

Calf immunity is not only affected by prenatal heat stress but is also influenced by heat stress during the postnatal period. Passive immunity is of importance in calves' health and survival. Compared with calves with adequate passive IgG transfer, calves with failure of passive transfer have up to a 4-fold increase in mortality (McEwan et al., 1970, McGuire et al., 1976). Donovan et al. (1986) examined the seasonal effect on passive transfer in calves raised in a subtropical climate and reported that calves born in summer had lower total serum protein compared with calves raised in other seasons. Importantly, the calves with lower total serum protein were more likely to die from pathogenic diseases within the first 14 weeks of age. This seasonal effect on passive immunity is partially explained by the reduced colostral IgG absorption by prenatal heat stress. However, the elevated ambient temperature at birth is also related to lower serum total protein of neonate (Donovan et al., 1986), suggesting impaired passive immune transfer by postnatal heat stress. Similarly, relative to calves housed without cooling, providing shade or evaporative cooling to calves immediately after birth improves the IgG transfer from colostrum to calves (Stott, et al., 1976). In addition to passive immunity, the humoral immunity of the calves is negatively affected by postnatal heat stress. Relative to calves housed in a thermo-neutral environment, neonatal calves under heat stress have lower circulating blood IgG concentrations (Kelley et al., 1982b), indicating that heat-stressed animals have a greater clearance of blood IgG or less endogenous IgG production, or a combination of both. Heat-stressed calves also display weaker delayed-type hypersensitivity responses to *Mycobacterium tuberculosis* (Kelley et al., 1982a), indicating a compromised cell-mediated immune response. As a result, heat stress increases mortality during the early life of calves (Stott et al., 1976; Stull et al., 2011).

Flies

Flies are widely distributed on dairy farms, especially during the warm temperatures. Flies represent a major economic burden to livestock production due to animal distress and discomfort, disease transmission, and pest control (Taylor et al., 2012). However, they are difficult to eradicate. For example, seasonal activity and intensity of stable flies commonly peak during the spring and early summer (Mullens and Meyer, 1987, Greene and Petersen, 1989). It has been estimated that issues related to stable flies resulted in approximately $360 million losses to the dairy industry in the U.S. In preweaned dairy calves, it was predicted that issues caused by flies resulted in a 6 kg reduction of body weight. However, there is a lack of research on how flies influence calf performance and the overall economic impact of flies to the dairy industry (Taylor et al., 2012).

Management practices to improve calf performance during summer

Heat abatement has been widely utilized on the dairy farm to improve performance, health, and overall welfare of the lactating animals. Compared with research conducted in mature animals, studies to explore heat abatement or nutritional approaches to reduce the negative impact of heat stress for preweaned dairy calves are few.

Housing management in the Southeast

Type of housing and bedding have shown to impact calf performance in summer. For example, during summer months, calves in hutches bedded with straw improved feed intake, weight gain, and health scores compared with calves in hutches on the sand bedding (Hill et al., 2011). In a study conducted in the subtropical climate of Florida, Peña et al. (2016) compared polyethylene hutches with wire hutches covered by a plywood, and reported that calves raised in the wire hutches with plywood cover had lower respiration rate and rectal temperature in the afternoon compared with those in polyethylene hutches. Type of hutches did not affect growth, but calves raised in polyethylene hutches showed less symptom of respiratory diseases (nasal discharge and coughing) and required less veterinary treatments compared with those raised in the wire hutches (Peña et al., 2016). It is important to note that calf housing design is not only dependent of the environmental condition but the economic status of a farm. For example, housing in the south varies from open wooden or wired hutches to sophisticated open barns with natural ventilation (Mark Hill, Provimi North America, Inc., personal communication).

Shade

Shade is an effective approach to reduce heat exchange by solar radiation of the calves during summer. For instance, providing supplemental shade cloth with 80% blockage to plastic hutches decreased hutch inside temperature by 1°C in the morning and 2 °C in the afternoon. (Spain and Spiers, 1996). Compared with shaded calves, calves in hutches without shade had higher skin temperature (2.45 °C) and respiration rates (10 breaths/minute) during the afternoon when environmental temperature was highest (Spain and Spiers, 1996). In a study conducted in Alabama, a shade cloth with 80% blockage positioned 1 meter above plastic hutches reduced hutch temperature by 1 C° and calf body temperature by 0.5 °C compared with calves raised in hutches without shade (Coleman et al., 1996). Stott et al. (1976) reported that providing shade to hutches immediately after birth increased serum IgG concentration at 2 and 10 days of age and reduced mortality rate during the first 20 d of age, suggesting improved passive transfer of immunity and overall health of preweaned calves. It is important to note that Coleman et al. (1996) reported an increase in the coliform count in the bedding materials collected from hutches under supplemental shade. These suggested that providing shade might increase the environmental bacterial load and emphasized the importance of good bedding management to prevent infection.

Aluminized hutch covers

A reflective aluminized low-density hutch cover is a technology to block solar radiation during the summer to alleviate heat stress for preweaned dairy calves. In an experiment conducted in two consecutive summers, Carter et al. (2014) found that providing the aluminized covers lowered hutch air temperature, and respiration rate and ear canal temperature of the calves during summer. However, the ADG of the calves was not improved. In contrast, in a study carried out in Colorado, preweaned calves placed in hutches with aluminized reflective covers were more likely to have diarrhea, increased abnormal ear score, and had similar ADG compared with calves housed in hutches without cover (Manriquez et al., 2018). Therefore, existing data do not provide sufficient evidence that reflective aluminized hutch covers significantly improve calf performance and health during summer.

Improving airflow

Adequate airflow is not only important to maintain air quality but also significant to reduce heat load of the heat-stressed calves. Elevating the back of the hutches may also benefit calves in the summer. It has been reported that elevating hutches at the back decreased airborne bacteria count by improving air turnover inside the hutch (Hill et al., 2011). Moreover, Moore et al. (2012) found that elevating straw bedded hutches using a concrete block (20×20×41 cm) at the back of hutches lowered calf respiration rate in the afternoon, mildly increased air flow and reduced carbon dioxide level inside the hutch. Unfortunately, calf weight gain was not assessed by Moore et al. (2012). More research is needed to assess the impact of hutch elevation on calf performance during summer.

When calves are raised under a barn, increasing forced air ventilation by fans has shown to improve calf performance during summer. In a study conducted during summer in southwest Ohio, calves were individually housed in wired hutches under a naturally ventilated barn and were either cooled with fans from 0800 to 1700 h or not. Compared with non-cooled calves, cooling reduced respiration rate, improved ADG by 19% and enhanced feed efficiency by 17% during the preweaned period. Similarly, in another experiment conducted in mid-Florida, Dado-Senn et al. (2020) evaluated the effect of cooling by fans on physiological responses and performance of preweaning calves raised on automatic calf feeders under an open-sided barn.

Although all calves experienced similar level of heat stress (THI = 78), calves cooled by fans had lower respiration rate, rectal temperature, skin temperature, and improved feed intake (Dado-Senn et al., 2020) compared with non-cooled calves. However, cooling had no impact on body growth or ADG (Dado-Senn et al., 2020).

Water availability

Prediction of water consumption in preweaned calves has not been well studied. In an experiment that enrolled 672 calves, Quigley (2001) reported that dry and liquid feed consumption and environmental conditions were the primary factors that influence water intake. Specifically, starter intake explained more than 60% of the variation of daily water consumption, and water intake increased exponentially as environmental temperature increased (Quigley, 2001, Chavez, 2011). Additionally, during the summertime, as evaporative cooling increases, water is lost as respiration rate and sweating rate increase. Therefore, sufficient water supply is of importance for calves to replenish body water for normal functions and effective evaporative cooling. However, the NAHMS survey reported that water was only offered to calves at 8.2 to 16.3 d after birth (USDA, 2010). Calves during the first 14 days of age are highly prone to develop mild to severe diarrhea causing dehydration. This will be amplified by heat stress leading to severe illness or death. Water quality is of importance as well. Regardless of season, cleaning and rinsing of water buckets daily have shown to reduce treatment for diseases and increase ADG compared with cleaning weekly or biweekly (Wiedmeier et al., 2005). Thus, clean and adequate water should be offered all time from immediately after birth to guarantee sufficient water consumption.

Calf starter management.

Reduced grain intake by heat stress limits energy available for maintenance and growth. Bateman et al. (2012) noted that grain and milk replacer intakes were the most important variables to predict growth in calves. Hence, encouraging grain intake provides an opportunity to improve growth during summer. However, related study is limited. In a study that examined different DM content of starter grain on calf performance during summer, water was added to starter grain to adjust the DM contents to 90, 75, and 50%. The grain intake of the calves increased linearly as the DM content decreased. This resulted in higher preweaning ADG of calves fed starter with lower DM content (Beiranvand et al., 2016). Other approaches to increase starter intake during summer will be of vital importance.

Increasing milk or MR feeding rate during summer

Because of the increased energy cost for maintenance and reduced starter intake, it is logical to hypothesize that increasing milk or milk replacer feeding rate in the summer would increase the energy intake, consequently improving growth. However, there is only limited research available to support this hypothesis. Hill et al. (2012) examined the different feeding levels of a MR containing 20% CP and 20% crude fat (20:20, as-fed basis) with different weaning age (28 and 42 d of age [**DOA**]) on calf performance during summer. In the first experiment, calves weaned at 28 DOA and received 0.44 or 0.55 kg/d during the 28 DOA or 0.66 kg/d for the first 14 d and 0.44 kg/d for the remaining 14 d. In the second experiment, calves were weaned at 28 or 42 DOA and fed 0.44 or 0.66 kg/d of a 20:20 MR. In both experiments, feeding more MR increased preweaning ADG but no differences observed on ADG after weaning. Interestingly, there were no differences on starter intake during the preweaning period.

These data suggest that increasing feeding rate of a 20:20 MR up to 0.66 kg/d can be a feasible method to improve growth during heat stress (Hill et al., 2012).

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CHAPTER 3

EFFECTS OF MILK REPLACER FEEDING LEVELS ON PERFORMANCE AND METABOLISM OF PREWEANED DAIRY CALVES DURING SUMMER[1]

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[1]Orellana Rivas, R. M., G. H. Komori, V. V. Beihling, T. N. Marins, J. K. Bernard, S. Tao. 2020. *Journal of Dairy Science*, 103(1): 313 – 324. Reprinted here with permission of publisher.

Abstract

The objective of this study was to evaluate the effect of milk replacer (MR) feeding programs on performance and metabolism during summer. At 3 d of age (DOA), calves were randomly assigned to 1 of 4 dietary treatments: control [CON; 0.55 kg dry matter (DM) of a 20% crude protein (CP) and 20% fat MR per day], intermediate (IL; 0.66 kg DM of a 26% CP and 17% fat MR per day), high (HL; 0.77 kg DM of a 26% CP and 17% fat MR per day), or aggressive (AL; 0.87 kg DM of a 26% CP and 17% fat MR per day). Calves were managed similarly and housed in individual polyethylene hutches using sand as a bedding material. Because 3 calves fed the AL diet developed abomasum bloating during the first 30 DOA, the AL treatment was terminated. Milk replacer (12.5% solids) was offered twice daily until 42 DOA, when MR was fed once daily to reduce its intake by 50%. Calves were weaned at 49 DOA and remained in hutches until 56 DOA. Calf starter and water were offered ad libitum. Ambient temperature and relative humidity in and outside the hutches were assessed hourly. Starter and MR intakes were recorded daily. Respiration rate and rectal temperature were determined 3 times each week. Body weight was measured at 3, 14, 28, 42, and 56 DOA. Plasma was collected at 5, 10, 14, 28, 42, 43, 45, 47, 49, 51, and 56 DOA for analysis of glucose, β-hydroxybutyrate, triglycerides, non-esterified fatty acids, urea nitrogen, and insulin concentrations. There were no treatment effects on starter intake, rectal temperature, or respiration rate. By 7 DOA, calves fed the IL and HL diets consumed the same amount of MR and a higher amount of MR than the CON calves. At wk 2, calves from all treatments had similar MR consumption before returning to the projected intake by design at wk 4. Calves fed the IL and HL treatments had similar body weight but were heavier than those fed the CON diet at wk 6, 7, and 8. Calves fed the IL and HL diets had similar average daily gain, which was higher than that of calves fed the CON diet.

There was no difference in plasma metabolites among treatments, but insulin concentration increased as milk allowance increased. In summary, feeding an intermediate level of MR during summer improved calf growth compared with the CON diet, but a higher MR allowance did not support further improvements in calf performance.

Key words: milk allowance, preweaned calf, heat stress

Introduction

Environment influences animals' performance, health, and related nutritional management. Compared with calves under temperate conditions, preweaned calves raised during summer have reduced ADG (Wiedmeier et al., 2006; Broucek et al., 2009; Chavez, 2011). Reasons for the poor performance of calves during summer are multifactorial and include pre- and postnatal heat stress, increased fly density, and wetter bedding due to increased rainfall (Broucek et al., 2009; Monteiro et al., 2016). Among these factors, the major factor limiting calf growth may be postnatal heat stress. Similar to mature cows, preweaned calves have increased body temperature and respiration rate when raised in an environment with elevated ambient temperatures (Gebremedhin et al., 1981; Hill et al., 2016a). The respiratory and cutaneous evaporative cooling of the calf is initiated when ambient temperature exceeds 20°C (Gebremedhin et al., 1981), above which energy utilization for cooling increases at the expense of other functions. This redistribution of energy increases energy required for maintenance and consequently reduces energy available for growth (Gebremedhin et al., 1981). Further, preweaned calves have decreased starter intake when exposed to summer conditions compared with those under thermoneutrality (Chavez, 2011). Reduced total energy intake results from lower starter intake, which, combined with the increase in energy cost for maintenance, decreases net energy available for growth. Consequently, preweaned calves raised during summer have lower ADG compared with those raised in cooled or temperate environments (Wiedmeier et al., 2006; Broucek et al., 2009). The stunted animal growth during summer may also lower future productivity. Soberon et al. (2012) reported that ADG during the preweaning period was positively correlated with a heifer's milk yield in first lactation. Reduced energy and nutrient consumption have implications for calf immunity and disease resistance as well. Compared with preweaned calves fed a low plane of nutrition of milk replacer (MR), calves fed a higher plane of nutrition had lower blood neutrophil activity before weaning (Obeidat et al., 2013; Ballou et al., 2015) but displayed stronger resistance to disease challenges during the postweaning period (Ballou et al., 2015; Sharon et al., 2019). Thus, management and nutrition strategies need to be developed to improve performance of calves raised during summer.

Management approaches, such as providing forced ventilation, have proven effective at improving calf growth in summer (Hill et al., 2011). However, cooling alone cannot completely abate heat stress. The diversity of calf housing options in the dairy industry also limits the utilization of heat abatement to improve growth. A recent national survey indicated that 37.9% of dairy operations in western and eastern regions of the United States used individual outside hutches to house preweaned calves (USDA–NAHMS, 2016), where forced ventilation is not effective. Similarly, individual hutches are the predominant housing option for preweaned calves in the southeastern region of the United States. Thus, additional nutrition strategies need to be developed to enhance calf growth during summer. Due to the higher energy requirement for maintenance and lower calf starter intake caused by heat stress, it is logical to hypothesize that increasing the quantity of MR fed to calves during summer would increase the dietary energy available for growth, thereby enhancing ADG. Hill et al. (2012) reported that increasing the feeding level of a traditional MR containing 20% CP and 20% fat (20:20, as fed basis) from 0.44

to 0.55 kg of DM/d during summer improved the ADG of preweaned calves without influencing calf starter intake. However, similar results were not observed during winter (Hill et al., 2012), suggesting that feeding more traditional MR to preweaned calves may improve animal growth in summer. Relative to the traditional 20:20 MR feeding regimen (0.44–0.55 kg/d), accelerated feeding programs that entail feeding large quantities of MR containing higher protein content have gained popularity due to the higher ADG of preweaned calves raised on this feed regimen (Khan et al., 2007). However, it is unknown whether increasing the feeding rate in an accelerated MR program during summer could improve calf growth.

Therefore, our hypothesis was that increasing the MR allowance of preweaned calves during summer would improve growth. The objective was to examine the effect of 4 MR feeding rates on calf growth performance and blood metabolites and insulin during summer.

Materials and methods

Animals and experimental design

The University of Georgia Institutional Animal Care and Use Committee approved the procedures and animal handling before trial initiation. The study was conducted at the Dairy Research Center of the University of Georgia–Tifton campus from June to October 2016. A total of 52 calves were enrolled in the experiment. Within 6 h after calving, calves were removed from their dam and fed 940 g of colostrum replacer containing at least 200 g of IgG (bovine IgG colostrum replacer; Land O'Lakes Inc., Arden Hills, MN). The day of birth was considered 1 d of age (DOA).

At 2 DOA, calves were fed 0.55 kg/d (DM basis) of a 20:20 MR (Provimi North America Inc., Brookville, OH) twice daily (0700 and 1600 h). At 3 DOA, calves were weighed and randomly assigned by sex and calving date to 1 of the 4 feeding programs: control [CON; n = 14

(8 heifers, 6 bulls); 0.55 kg DM of 20:20 MR (Provimi North America Inc.) per day], intermediate [IL; n = 13 (7 heifers, 6 bulls); 0.66 kg DM of 26% CP and 17% fat (as-fed basis; 26:17) MR (Provimi North America Inc.) per day], high [HL; n = 13 (6 heifers, 7 bulls); 0.77 kg DM of 26:17 MR per day], and aggressive [AL; n = 12 (6 heifers, 6 bulls); 0.87 kg DM of 26:17 MR per day]. Both MR products were medicated with lasalocid and comprised the same ingredients, including dried whey, dried milk protein, whey protein concentrate, dry skim milk, and vegetable and animal fat. Three calves fed the AL diet developed abomasal bloating at 10, 10, and 29 DOA, and 2 died. No abomasal bloating was observed in other treatments. Consequently, the AL treatment was terminated. All calves were managed similarly and housed in individual polyethylene hutches without shade. During the experiment, the door of the hutches was oriented to the east, and the windows in the back and on the ridge of the hutch were opened for ventilation. Sand was used as the bedding material and was cleaned twice each day and replaced once weekly.

Feeding, intake, and growth measures

Milk replacer was reconstituted with warm water to 12.5% solids and offered to calves twice daily at 0700 and 1600 h. Equal amounts were fed each feeding until 42 DOA, when MR allowance was reduced 50% and fed once daily (0700 h). Calves were initially fed by bottles and were trained to drink from buckets at 4 DOA. Calves were weaned at 49 DOA and remained in hutches until 56 DOA. The MR intake was calculated by subtracting the amount refused from the amount offered. Textured calf starter (Godfrey's Warehouse Inc., Madison, GA) was provided ad libitum starting at 2 DOA, and the intake was recorded daily. From 43 DOA, when the MR allowance was reduced, 227 mg of a coccidiostat (amprolium, Corid 1.25% Crumbles; Provimi North America Inc.) was supplemented daily until calves completed the experiment.

Representative samples of MR and calf starter were collected once each week and pooled monthly for chemical analysis (Table 3.1) at Cumberland Valley Analytical Services (Waynesboro, PA). Water was offered ad libitum throughout the entire experiment. To evaluate the growth, BW, body length, withers height, heart girth, and hip height were measured at 3, 14, 28, 42, and 56 DOA, approximately 3 h after morning feeding.

Environment, rectal Temperature, respiration rate, fecal scoring, and scour incidence

The ambient temperature and relative humidity in and outside the hutch were measured every 15 min by Hobo Pro Series Temp probes (Onset Computer Corporation, Pocasset, MA) during the entire experiment. For outside temperatures, a probe was hung on the fence adjacent to the hutches. A probe was hung in the middle of an empty hutch approximately 1 m above the ground to measure temperatures inside the hutch. Rectal temperature and respiration rate were assessed 3 times each week (Monday, Wednesday, and Friday) at 1430 h. Rectal temperature was measured using a thermometer (20-s digital thermometer, 144-920-000, ReliOn; Mabis Healthcare Inc., Waukegan, IL), and respiration rate was assessed by counting flank movement for 1 min. Fecal score was recorded twice daily (0730 and 1630 h) throughout the experiment according to the calf health scoring chart developed by the School of Veterinary Medicine, University of Wisconsin–Madison (2011). A scouring calf was diagnosed based on the appearance of loose feces, reduced intake, and dehydration and was treated with 3 doses of ceftiofur sodium (Naxcel; Zoetis Services LLC, Parsippany, NY) and 1 dose of flunixin meglumine (Banamine; Merck Animal Health Intervet Inc., Madison, NJ). The MR allowance during the afternoon feeding was replaced with electrolyte (Re-Sorb; Zoetis Services LLC) for 3 to 5 d depending on whether the calf was recovered.

Blood sample collection and analyses

Blood samples were collected via jugular venipuncture into sodium-heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) at 5, 10, 14, 28, 42, 43, 45, 47, 49, 51, and 56 DOA at 1100 h and immediately placed on ice. Samples were centrifuged at 2,619 × g at 4°C for 30 min to collect plasma. Plasma concentrations of glucose (autokit glucose; Wako Chemicals USA Inc., Richmond, VA), nonesterified fatty acids [HR series NEFA-HR(2); Wako Chemicals USA Inc.], BHB (autokit 3-HB; Wako Chemicals USA Inc.), triglyceride (L-type triglyceride M; Wako Chemicals USA Inc.), urea nitrogen (urea nitrogen kit; Pointe Scientific Inc., Canton, MI), and insulin (bovine insulin ELISA; Mercodia AB, Uppsala, Sweden) were determined using commercially available kits; the inter- and intra-assay coefficients of variation were 9.5 and 4.2%, 5.8 and 5.8%, 8.0 and 3.6%, 16.7 and 7.0%, 7.1 and 6.7%, and 13.5 and 3.7%, respectively.

Statistical analyses

Because the AL treatment was terminated, only data collected from calves enrolled in the CON, IL, and HL treatments were included in the statistical analyses. The UNIVARIATE procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) was used to summarize the ambient temperature and relative humidity, and the means ± standard deviation are reported. Repeated-measures data, including intake data (MR, starter, total DM, fat, CP, ME), BW, ADG, weekly fecal score, and plasma concentrations of metabolites and insulin, were analyzed using the MIXED procedure of SAS 9.4. The model included fixed effects of treatment, sex, time, and their interactions with calf (treatment) as the random effect, and the least squares means ± standard error of the mean were reported. The incidence of scours was analyzed using the LOGISTIC procedure of SAS 9.4, and the day of the first incidence of scours was analyzed using the GLM procedure of SAS 9.4. The models included treatment and sex, and the least squares means ± standard error of the mean were reported. Simple regression using the GLM procedure with the solution function of SAS 9.4 was used to determine the relationships between ADG and intakes from 3 to 49 DOA.

Results

Nutrient contents of MR and calf starter are reported in Table 1. The CP and fat contents (% of DM) for 20:20 MR, 26:17 MR, and calf starter were 20.5 and 22.8%, 27.5 and 18.7%, and 18.6 and 3.3%, respectively. During the experiment, the average ambient temperature and relative humidity were 26.0 ± 5.8°C (mean ± SD) and 75.2 ± 19.9% inside the hutch and 25.4 ± 4.0°C and 79.2 ± 16.3% outside the hutch, respectively. From 1000 to 1800 h, the ambient temperature was higher, but the relative humidity was lower inside the hutch than outside (Figure 3.1). Milk replacer feeding rates or treatment × time interaction had no ($P \geq 0.28$) effect on rectal temperature or respiration rate of the calves (Table 3.2). However, treatment tended ($P \leq 0.10$) to affect fecal score and the incidence of scours, as calves fed the HL diet had greater ($P \leq 0.05$) fecal score during the first 4 wk of life and higher ($P \leq 0.05$) incidence of scours compared with calves fed the CON diet; values for calves fed the IL diet were intermediate (Table 3.2).

There was a treatment × time interaction ($P < 0.01$) for MR intake (Table 3.3; Figure 3.2A). At wk 1 of age, calves fed the IL and HL diets consumed the same amount ($P = 0.76$) of MR and consumed a higher ($P < 0.01$) amount than calves fed the CON diet. At wk 2, calves from all treatments had similar ($P \geq 0.18$) MR consumption, mainly due to a higher occurrence of scours, when MR was replaced with electrolytes during the afternoon feeding. From wk 3 to 7 of age, calves fed the HL diet consumed more MR than calves fed the CON diet; values for calves fed the IL diet were intermediate ($P < 0.01$; Figure 3.2A). Treatment or treatment × time interaction had no effect on calf starter intake or total DMI ($P \geq 0.13$; Table 3.3). However, there were treatment × time interactions ($P \leq 0.05$) for intake of CP, fat, and ME (Table 3.3; Figure 3.2). From wk 1 to 6, intake of CP was similar ($P \geq 0.26$) in calves fed the HL and IL diets and was higher ($P \leq 0.05$) than that in calves fed the CON diet (Figure 3.2B). Fat intake was greater ($P = 0.03$) for CON calves compared with IL or HL calves at wk 2 of age. However, from wk 4 to 6, calves fed the CON and IL diets had similar ($P \geq 0.62$) fat intake, which was lower ($P \leq 0.01$) than that observed for calves fed the HL diet (Figure 3.2C). From wk 3 to 6 of age, calves fed the HL diet consumed more ($P < 0.05$) ME compared with calves fed the CON diet; values for calves fed the IL diet were intermediate (Figure 3.2D).

There was no difference ($P = 0.11$) between treatments on BW, but calves fed the CON diet had lower ($P < 0.01$) BW compared with calves fed the HL and IL diets at 42, 49, and 56 DOA (treatment × time: $P < 0.01$; Table 3.4; Figure 3.3). Compared with those fed the CON diet, calves fed the HL and IL diets had greater ADG ($P = 0.05$) and overall gain in BW, heart girth, and hip height ($P \leq 0.02$) from 3 to 56 DOA, but no differences ($P > 0.10$) were observed between calves fed the HL and IL diets (Table 3.4). There were no differences between treatments in withers height gain from 3 to 56 DOA, but calves fed the IL and HL diets had greater ($P = 0.05$) withers height gain from 28 to 56 DOA compared with calves fed the CON diet (Table 3.4).

Milk replacer feeding rates had no ($P \geq 0.16$) effect on ADG per kilogram of CP or fat intake. Compared with CON calves, calves fed the IL and HL diets had greater ($P \leq 0.05$) ADG/DMI and ADG/ME intake, but no differences were observed between calves fed IL and HL (Table 3.3). The ADG was correlated ($P < 0.01$) with total intake of DM, CP, fat, and ME, and the coefficient of determination was 0.58, 0.63, 0.18, and 0.69, respectively (Figure 3.4).

There were no differences ($P \geq 0.26$) among treatment or interactions for plasma glucose, nonesterified fatty acids, BHB, and triglyceride. Plasma insulin concentrations were highest ($P < 0.01$) for HL, intermediate for IL, and lowest for CON (Table 3.5). A treatment × time interaction ($P = 0.05$) was observed for plasma urea nitrogen concentration, mainly due to the effect of time within each treatment because no differences were observed between treatments at each time points.

Discussion

Calves were exposed to elevated ambient temperature and relative humidity throughout the current trial. The rectal temperature and respiration rate of calves averaged 39.6°C and 75 breath/min, respectively, which is above the normal body temperature (38.5°C) of a calf under thermoneutral conditions (Collier et al., 2019) but did not differ among treatments. These data suggest that calves enrolled in this experiment experienced heat stress throughout the trial. Interestingly, the ambient temperature inside the hutch was 1.5 to 3.8°C higher than that outside from 1000 to 1800 h during the day. This suggests that the enclosed hutch accumulates heat during the hottest time of day and indicates a need for interventions to reduce heat accumulation. In a relatively dry environment (relative humidity ranges from 15 to 70%), elevating the back of the hutches by 20 cm slightly reduced the internal ambient temperature of the hutch by 0.12°C compared with the external ambient temperature and decreased calf respiration rate in the afternoon (Moore et al., 2012). In the current study, the windows located in the back and on the ridge of the hutch were fully opened to improve ventilation, but the back of the hutches was not elevated. In a study by Spain and Spiers (1996), providing an 80% solar radiation blockage shade cloth over plastic hutches reduced the temperature inside hutches by 2.3°C compared with hutches without shade (29.7 vs. 32.0°C, respectively). Alternatively, the door of the hutches can be oriented to the east to maximize the shade area outside the hutches in the late afternoon, when the ambient temperature inside the hutches is higher than that outside.

The objective of this study was to investigate whether increasing the feeding rate of MR could improve growth of preweaned calves during summer heat stress. Three out of 12 calves enrolled in the AL treatment experienced abomasal bloating during the first 30 DOA, and 2 died (17% mortality rate). No abomasal bloating was observed in calves fed the other treatments. The high incidence of abomasal bloating observed for AL was unexpected and inconsistent with previous reports. Using a similar MR product (26:17), no abomasal bloating was reported for calves fed 0.87 kg/d or more twice daily in a temperate environment (average ambient temperature: 21°C; Chapman et al., 2016; Hill et al., 2016b). This surprising finding suggests that feeding a large quantity of milk twice daily in an extreme heat stress environment, as is often observed during summer in the southeastern region of the United States, delays MR abomasal emptying rate, which resulted in excessive gas production and abomasal bloating (Burgstaller et al., 2017). Heat stress is known as a limiting factor for gastric motility in dairy cattle. Under summer or environment conditions characterized by elevated temperature, lactating dairy cows and prepubertal heifers have slower rate of passage (McDowell et al., 1969; Nonaka et al., 2008), which enhances nutrient digestibility but limits feed intake. The effect of heat stress on calf gastric motility and abomasal emptying has not been reported (Burgstaller et al., 2017). In addition to volume, osmolality of the milk influences abomasal emptying rate (Burgstaller et al., 2017). Elevation of osmolality of the milk by mixing with electrolytes significantly delays the abomasum emptying rate (Constable et al., 2009). The osmolality of MR used in the current experiment was roughly estimated by adding the osmolality of the lactose and microminerals and averaged 333.98 and 332.10 mOsm/kg for the 20:20 and 26:17 MR, respectively. The reasons for greater fecal score and increased incidence of scours of calves fed more milk relative to CON calves are unclear but may suggest a disturbance of digestion in calves fed large quantities of MR in the summer.

Among the 3 remaining treatments, MR intake reached the expected levels only after 4 wk of age, especially for IL and HL calves. This observation contradicts previous studies conducted in temperate environments (Terré et al., 2006; Chapman et al., 2016; Hill et al., 2016b; MacPherson et al., 2016), which reported that calves were able to consume large quantities of MR (>0.77 kg/d) twice daily after wk 2 of age, suggesting that calves raised in heat stress conditions not only have reduced starter intake but may also have decreased MR intake when a large amount of MR is fed twice daily. The biological mechanism of the reduced MR intake is unknown but presumably attributed to the delayed gastric motility and abomasal emptying. In prepubertal heifers and adult cattle, heat stress reduces gastric motility, which inhibits DMI (McDowell et al., 1969; West, 2003; Nonaka et al., 2008). No treatment effect was observed for starter intake, which is contradictory to previous studies in which feeding more MR in a temperate environment reduced starter intake (Terré et al., 2006; Chapman et al., 2016; Hill et al., 2016b); however calves fed the HL diet did not consume as much MR as allotted.

Feeding accelerated MR (IL and HL) increased ADG and skeletal growth compared with feeding the CON diet, confirming the advantage of accelerated MR over conventional MR for improving growth performance (Khan et al., 2007). Although they consumed more MR and total ME, HL calves had similar ADG and gain in skeletal growth during the preweaning period relative to IL calves. Feeding large quantities of milk may impede nutrient digestibility of calf starter. The effect of milk feeding rate on apparent total-tract nutrient digestibility of calf starter in temperate environments was examined previously using either direct (total fecal collection; Terré et al., 2007) or indirect (indigestible marker; Chapman et al., 2016; Hill et al., 2016b) methods. Studies suggested that feeding large quantities of MR twice daily reduced digestibility of DM, OM, fat, CP, and NDF provided by calf starter, primarily due to delayed rumen development as a result of lower starter intake (Terré et al., 2007; Chapman et al., 2016; Hill et al., 2016b). In the current study, no difference in starter intake was observed between calves fed HL and IL, and all calves had similar plasma levels of BHB during the entire experimental period, suggesting similar rumen development. Compared with preweaned calves fed 0.66 kg DM of 26:17 MR/d, similar to the MR in the current study, calves fed 1.31 kg of MR/d had higher digestibility of DM, OM, fat, and CP provided by MR at wk 3 of age (Hill et al., 2016b), indicating improved MR digestion. In veal calves, feeding MR at 2.5 × ME for maintenance (MEm) resulted in similar MR nutrient digestibility as in calves fed MR at 1.5 × MEm (van den Borne et al., 2006). Therefore, it is unlikely that the altered nutrient digestibility of MR or calf starter is the reason for the discordance between MR intake and ADG observed in the current study. However, future research is needed to examine the effect of MR feeding rate on digestibility of nutrients provided by MR and calf starter during summer.

Relative to veal calves fed MR at 1.5 × MEm, increasing the feeding rate to 2.5 × MEm increased heat production from the oxidation of AA and carbohydrates on the BW basis, suggesting increased energy loss and lower nutrient utilization for growth (van den Borne et al., 2006, 2007). The inefficient utilization of energy and nutrients by feeding more MR may be due to gastrointestinal hypertrophy to cope with the large meal sizes and delayed abomasal emptying (MacPherson et al., 2016) and to the increase in temporal plasma AA concentrations that exceed the requirement for protein retention, resulting in AA oxidation (van den Borne et al., 2006, 2007). Delayed abomasal emptying may also increase abomasal fermentation of nutrients, especially carbohydrates (Burgstaller et al., 2017). These will result in a net loss of energy and nutrients before absorption in the small intestine. Thus, it seems that large meal size and delayed abomasal emptying are potential mechanisms limiting nutrient utilization of calves fed large quantities of milk with limited feeding frequency (e.g., twice daily). Under a temperate environment and normal gastric functions, calf abomasal emptying will be delayed to a certain extent when feeding a large quantity of milk per meal (MacPherson et al., 2016). Delayed abomasal empting is considered a mechanism to regulate large influxes of nutrients into the small intestine and has minimal effect on animal growth (van den Borne et al., 2006; MacPherson et al., 2016). However, during summer, abomasal emptying may be further delayed by heat stress, which may exaggerate the loss of energy and nutrients, causing lower growth.

In addition to energy and nutrient utilization, MR feeding rate and frequency influence calf growth through altered glucose metabolism. In veal calves, long-term feeding with large quantities of MR twice daily is related to hyperglycemia, hyperinsulinemia, and insulin resistance in peripheral tissues (Doppenberg and Palmquist, 1991; Hostettler-Allen et al., 1994). The reduced insulin sensitivity results from less-abundant insulin receptors on peripheral tissues due to a negative feedback mechanism of prolonged elevation of plasma insulin (Hugi et al., 1998). In dairy calves, studies reported that increasing the MR feeding rate increased the insulin response after a glucose tolerance test but did not alter the glucose clearance of a preweaned calf (Bach et al., 2013; Yunta et al., 2015; MacPherson et al., 2016). Similarly, we observed that plasma glucose concentration was not influenced by MR intake during summer but that insulin concentration was increased as the feeding rate increased (CON < IL < HL). These data indicate that feeding large quantities of MR increases pancreatic sensitivity to release insulin and possibly results in reduced insulin action on peripheral tissues (e.g., muscle and adipose tissue; Bach et al., 2013; Yunta et al., 2015; MacPherson et al., 2016). Heat stress may also influence glucose and insulin metabolism of the calf. Previous studies (Tao et al., 2014; Monteiro et al., 2016) observed that maternal heat stress during the last 45 d of gestation increased glucose clearance after glucose tolerance test of the preweaned calf without enhancing insulin sensitivity on peripheral tissues (Monteiro et al., 2016), suggesting an increase in glucose utilization by insulin-independent tissues. Therefore, animals raised under heat stress conditions may have lower efficiency of glucose utilization for growth, which may be exaggerated by feeding large amounts of milk.

In temperate environments, increasing MR feeding frequency from twice to 3 or 4 times daily improves calf growth before weaning (van den Borne et al., 2006, 2007; Sockett et al., 2011; MacPherson et al., 2016). Further, increasing MR feeding frequency has been proven to accelerate abomasal emptying (MacPherson et al., 2016), improve energy and nutrient utilization (van den Borne et al., 2006, 2007), and potentially alter glucose partitioning toward peripheral tissues in preweaned calves (MacPherson, 2016). Therefore, more frequent feeding may be implemented when a large quantity of MR is fed during summer to overcome the potential limitation of delayed abomasal emptying, losses of energy and nutrients, and glucose metabolism altered by large meal size and heat stress. Related studies are not available but deserve further investigation.

In addition to increased MR allowance to increase the energy intake of a calf during summer, the other strategy is to increase the fat content of MR. A previous study (van den Borne et al., 2007) indicated that preruminant calves require glucose as a fuel rather than fat, which is primarily deposited into adipose tissue. In the current study, regardless of treatment, the ADG of calves from 3 to 49 DOA had the lowest correlation with total fat intake compared with total intake of DMI, CP, and ME. Calves fed the CON diet ingested similar amounts of fat relative to calves fed the IL diet but had lower ADG, further indicating that increasing MR fat content may have minimal effect on calf growth during summer. The ADG of calves had the highest correlation with total ME intake, suggesting the importance of increasing energy intake for increased ADG during summer. This, however, may not be achieved by increasing the MR fat content.

It is important to recognize that postnatal heat stress is not the only environmental cue that influences calf performance during summer. Previous studies (Monteiro et al., 2016; Laporta et al., 2017) indicated that maternal heat stress during the dry period negatively influenced growth performance of the calves and affected glucose metabolism during the preweaning period. In the current study, calves were balanced by calving date before enrolling into the treatments, but the possibility that there was an interaction between maternal heat stress and postnatal nutritional management on calf growth cannot be excluded and deserves further investigation. Additionally, the long day photoperiod has positive effects on starter intake and ADG before weaning relative to the short-day photoperiod (Osborne et al., 2007). However, the longer day length during summer cannot override the negative effect of heat stress on calf growth because of a lower ADG observed during summer (Wiedmeier et al., 2006; Broucek et al., 2009).

Conclusion

During summer, calves experience reduced growth partially due to altered energy metabolism and reduced starter intake because of heat stress. The result of the current study indicated that feeding an accelerated 26:17 MR at 0.66 kg of DM/d twice daily improved growth compared with feeding a conventional 20:20 MR at 0.55 kg of DM/d during summer. However, increasing the MR allowance to 0.77 kg of DM/d failed to improve growth further, and higher levels of MR feeding were related to increased incidence of abomasal bloating. These results suggest that feeding large quantities of MR with limited frequency during summer may be associated with delayed abomasal emptying rate due to large meal size and heat stress, which prevents effective energy utilization for growth. Future research needs to focus on approaches (i.e., increased feeding frequency) that could accelerate abomasal emptying and increase energy utilization of the calves when feeding a large quantity of MR during summer.

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Table 3. 1: Nutrient content (mean ± SD) of milk replacers (MR) containing either 20% CP and 20% fat (as-fed basis; 20:20) or 26% CP and 17% fat (as-fed basis; 26:17) and calf starter

Item, % of DM unless noted	**20:20 MR (n = 5)**	**26:17 MR (n = 5)**	**Calf starter (n = 5)**
DM, %	95.68 ± 0.84	95.12 ± 0.61	87.20 ± 2.28
CP	20.50 ± 0.66	27.54 ± 1.40	18.64 ± 1.45
Fat	22.82 ± 0.47	18.68 ± 1.33	3.33 ± 0.32
Lactose[1]	50.55 ± 0.62	47.45 ± 2.18	—
ADF	—	—	12.46 ± 0.50
Ash-free NDF	—	—	22.16 ± 1.16
NFC	—	—	47.18 ± 2.33
Ash	6.13 ± 0.40	6.33 ± 0.52	8.71 ± 1.40
ME, [1] Mcal/kg of DM	4.90 ± 0.03	4.80 ± 0.09	2.84 ± 0.08
Calcium	0.47 ± 0.01	0.53 ± 0.06	1.13 ± 0.28
Phosphorus	0.47 ± 0.01	0.54 ± 0.03	0.50 ± 0.02
Magnesium	0.07 ± 0.00	0.08 ± 0.01	0.30 ± 0.04
Potassium	1.41 ± 0.05	1.43 ± 0.06	1.68 ± 0.09
Sodium	0.46 ± 0.02	0.53 ± 0.14	0.28 ± 0.12
Osmolality, [2] mOsm/kg	333.98 ± 6.98	332.10 ± 24.47	—
Iron, mg/kg of DM	70.00 ± 25.77	79.20 ± 58.30	249.80 ± 22.22
Manganese, mg/kg of DM	33.80 ± 12.68	17.20 ± 3.27	96.00 ± 17.51
Zinc, mg/kg of DM	60.40 ± 11.06	28.40 ± 14.12	130.80 ± 20.64
Copper, mg/kg of DM	12.00 ± 7.38	24.00 ± 41.42	20.40 ± 2.07

[1]Calculated according to NRC (2001). 2 Roughly estimated according to the following equation: osmolality, mOsm/kg = [lactose, mmol/L] + [sodium, mmol/L] + [potassium, mmol/L] + [magnesium, mmol/L] + [calcium, mmol/L] + [phosphorus, mmol/L] (Constable et al., 2009; Wilms et al., 2019).

Table 3. 2: Rectal temperature, respiration rate, fecal scores, incidence of scours, and the day of the first incidence of scour of calves fed experimental diets starting from d 3 of age[1]

	Treatment [2]				***P*-value**		
Item	**CON**	**IL**	**HL**	**SEM**	**Treatment**	**Time**	**Treatment × time**
Rectal temperature, °C	39.63	39.55	39.64	0.06	0.58	<0.01	0.50
Respiration rate, breaths/min	73.39	74.11	76.51	1.96	0.51	<0.01	0.28
Fecal score wk 1–4	0.69 [a]	0.87 [ab]	0.97 [b]	0.09	0.10	<0.01	0.55
Fecal score wk 5–8	0.21	0.18	0.15	0.04	0.52	0.22	0.84
Scour incidence, % (no.)	28.6 [a] (4/14)	61.5 [ab] (8/13)	69.2 [b] (9/13)	—	0.07	—	—
Day of the first scour, d	13.1	9.7	15.5	2.16	0.12	—	—

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]All calves were housed in individual polyethylene hutches during the summer and fed twice daily.

[2]CON = 0.55 kg DM of a 20% CP and 20% fat milk replacer per day; IL = 0.66 kg DM of a 26% CP and 17% fat milk replacer per day; HL = 0.77 kg DM of a 26% CP and 17% fat milk replacer per day.

Table 3. 3: Intake and efficiency of calves fed experimental diets starting from d 3 of age[1]

Item	Treatment [2]			SEM	*P*-value		
	CON	IL	HL		Treatment	Time	Treatment × time
Milk replacer, kg/d	0.466	0.555	0.615	0.010	<0.01	<0.01	<0.01
Starter, kg/d	0.413	0.417	0.393	0.049	0.93	<0.01	0.99
DMI, kg/d	0.827	0.905	0.929	0.046	0.25	<0.01	0.13
CP, kg/d	0.162	0.212	0.221	0.010	<0.01	<0.01	0.05
Fat, kg/d	0.109	0.105	0.114	0.003	0.20	<0.01	<0.01
ME, Mcal/d	3.199	3.527	3.687	0.152	0.07	<0.01	0.01
ADG/DMI, g/g	0.338 [a]	0.441 [b]	0.446 [b]	0.030	0.02	<0.01	0.78
ADG/CP, g/g	1.732	1.921	1.903	0.140	0.56	<0.01	0.99
ADG/fat, g/g	4.345	5.646	5.389	0.504	0.16	<0.01	0.77
ADG/ME, g/Mcal	92.2 [a]	117.4 [b]	115.5 [b]	8.3	0.05	<0.01	0.92

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]All calves were housed in individual polyethylene hutches during the summer and fed twice daily.

[2]CON = 0.55 kg DM of a 20% CP and 20% fat milk replacer per day; IL = 0.66 kg DM of a 26% CP and 17% fat milk replacer per day; HL = 0.77 kg DM of a 26% CP and 17% fat milk replacer per day.

Table 3. 4: Growth performance of calves fed experimental diets starting from 3 d of age[1]

	Treatment [2]				*P*-value		
Item	**CON**	**IL**	**HL**	**SEM**	**Treatment**	**Time**	**Treatment × time**
BW, kg	45.29	49.79	49.34	1.63	0.11	<0.01	<0.01
ADG, kg/d	0.34 [a]	0.47 [b]	0.46 [b]	0.04	0.05	<0.01	0.26
BW gain, kg							
d 3–28	4.81 [a]	7.71 [b]	8.68 [b]	0.84	0.01	—	—
d 28–56	11.83 [a]	16.77 [b]	16.79 [b]	1.78	0.09	—	—
d 3–56	16.57 [a]	24.46 [b]	25.49 [b]	2.12	0.01	—	—
Heart girth gain, cm							
d 3–28	1.83 [a]	4.76 [b]	5.21 [b]	0.62	<0.01	—	—
d 28–56	6.89	7.59	8.14	0.94	0.64	—	—
d 3–56	8.65 [a]	12.36 [b]	13.36 [b]	1.12	0.01	—	—
Withers height gain, cm							
d 3–28	3.32	3.27	3.70	0.48	0.80	—	—
d 28–56	3.66 [a]	4.88 [b]	4.82 [b]	0.36	0.04	—	—
d 3–56	6.97	8.14	8.52	0.55	0.12	—	—
Hip height gain, cm							
d 3–28	3.01	2.77	3.54	0.46	0.49	—	—
d 28–56	3.38 [a]	4.45 [ab]	5.10 [b]	0.42	0.02	—	—
d 3–56	6.30 [a]	7.22 [ab]	8.63 [b]	0.57	0.02	—	—
Body length gain, cm							
d 3–28	3.64	4.51	4.39	0.57	0.50	—	—
d 28–56	4.63	5.71	5.42	0.50	0.31	—	—
d 3–56	8.23 [a]	10.21 [b]	9.81 [ab]	0.60	0.06	—	—

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]All calves were housed in individual polyethylene hutches during the summer and fed twice daily.

[2]CON = 0.55 kg DM of a 20% CP and 20% fat milk replacer per day; IL = 0.66 kg DM of a 26% CP and 17% fat milk replacer per day; HL = 0.77 kg DM of a 26% CP and 17% fat milk replacer per day.

Table 3. 5: Select plasma metabolites and insulin concentrations of calves fed experimental diets starting from d 3 of age[1]

	Treatment [2]				*P*-value		
	CON	**IL**	**HL**		**Treatment**	**Time**	**Treatment**
Item				**SEM**			**× time**
Glucose, mg/dL	79.4	81.3	84.6	2.6	0.38	<0.01	0.29
Insulin, µg/L	0.30 [a]	0.40 [b]	0.53 [c]	0.04	<0.01	<0.01	0.14
Nonesterified fatty acids, µEq/L	183.7	177.7	180.0	7.0	0.83	<0.01	0.63
BHB, mg/dL	0.84	0.88	0.83	0.08	0.90	<0.01	0.26
Triglyceride, mg/dL	11.6	11.3	11.2	1.3	0.97	<0.01	0.29
Urea nitrogen, mg/dL	10.8	13.3	11.1	1.5	0.49	<0.01	0.05

[a-c]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]All calves were housed in individual polyethylene hutches during the summer and fed twice daily.

[2]CON = 0.55 kg DM of a 20% CP and 20% fat milk replacer per day; IL = 0.66 kg DM of a 26% CP and 17% fat milk replacer per day; HL = 0.77 kg DM of a 26% CP and 17% fat milk replacer per day.

Figure 3. 1: Ambient temperature and relative humidity inside and outside the hutches

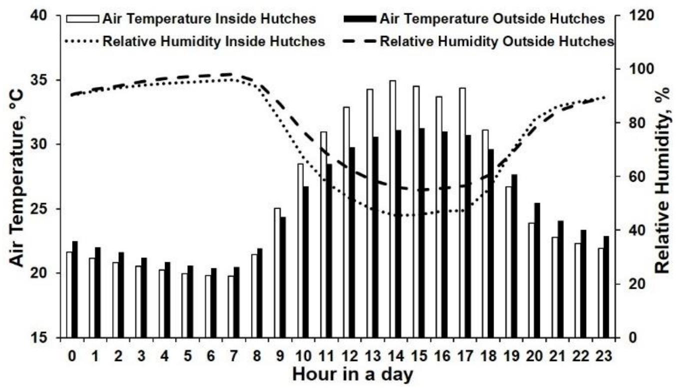


Figure 3. 2: The intakes of milk replacer (MR), CP, fat, and ME of calves fed experimental diets starting at d 3 of age.CON = 0.55 kg DM of a 20% CP and 20% fat milk replacer per day; IL = 0.66 kg DM of a 26% CP and 17% fat milk replacer per day; HL = 0.77 kg DM of a 26% CP and 17% fat milk replacer per day. Error bars represent SEM. **P ≤ 0.01, *P ≤ 0.05, †P ≤ 0.10.

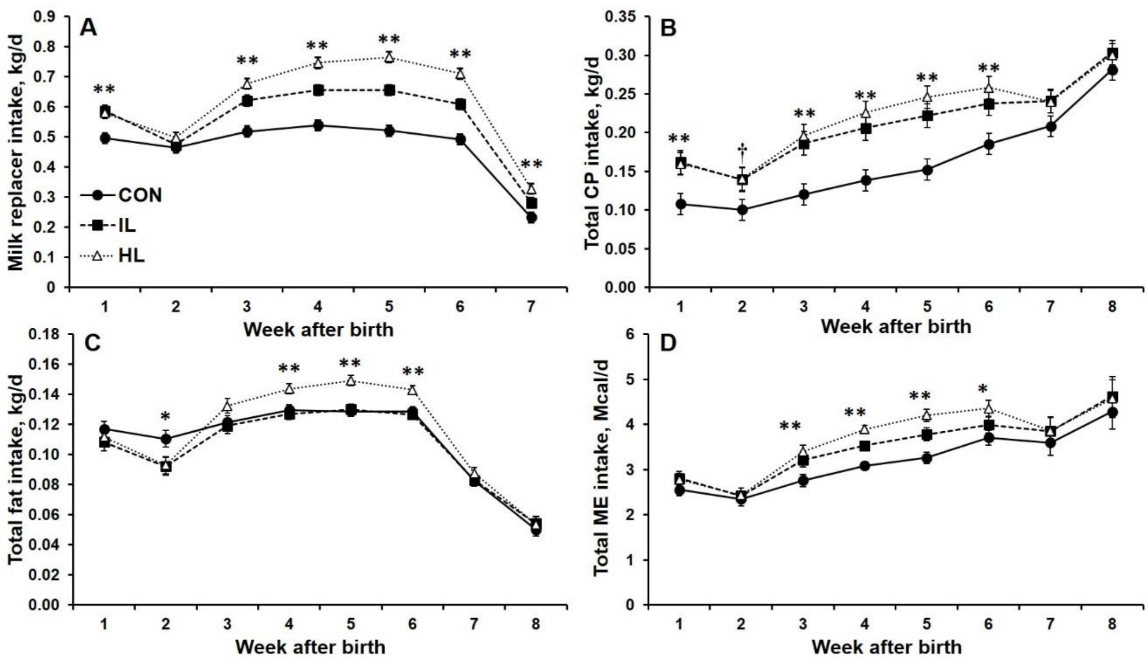


Figure 3. 3: Body weight of calves fed experimental diets starting from d 3 of age. CON = 0.55 kg DM of a 20% CP and 20% fat milk replacer per day; IL = 0.66 kg DM of a 26% CP and 17% fat milk replacer per day; HL = 0.77 kg DM of a 26% CP and 17% fat milk replacer per day. Error bars represent SEM. **P ≤ 0.01, *P ≤ 0.05, †P ≤ 0.10.

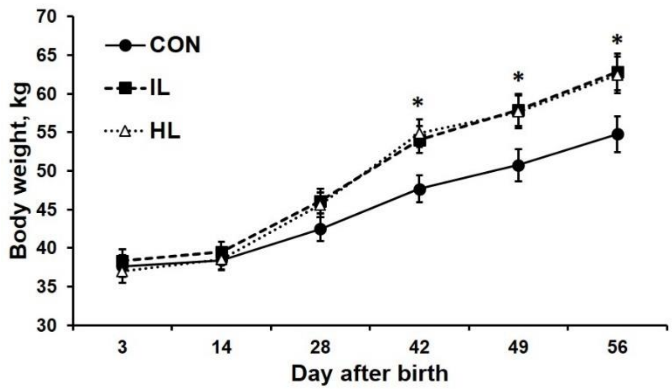
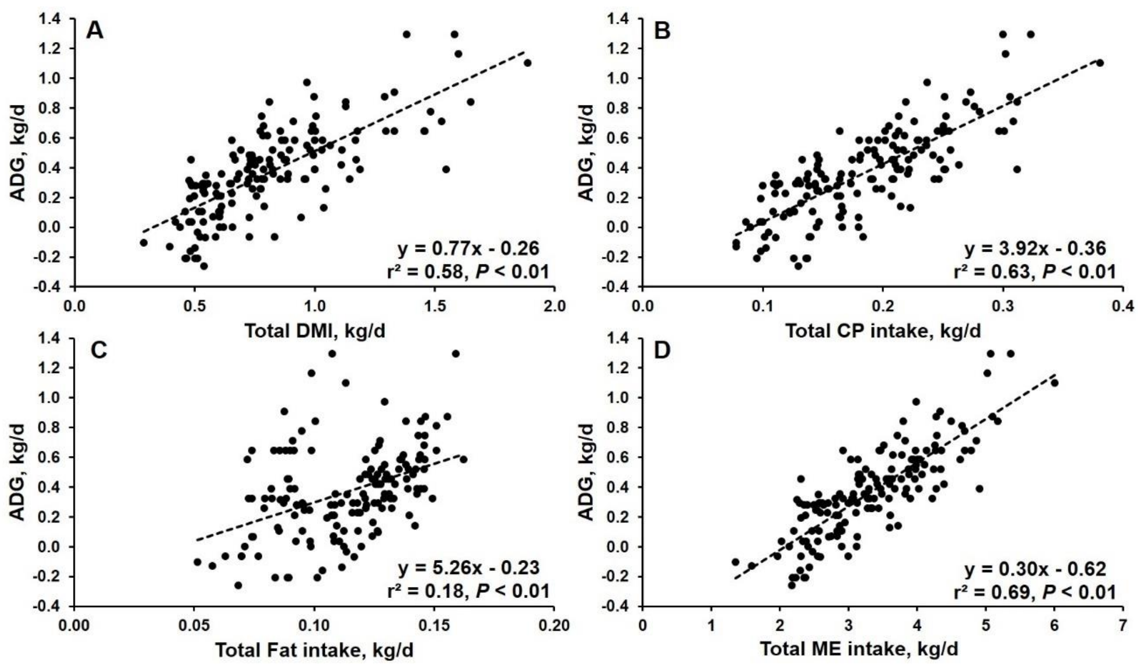


Figure 3. 4: The correlation between ADG and the total intake of DM, CP, fat, and ME of calves from 3 to 49 d of age.



CHAPTER 4

EFFECTS OF MILK REPLACER FEEDING RATE AND FREQUENCY ON PERFORMANCE, ABOMASAL EMPTYING, NUTRIENT DIGESTION AND METABOLISM OF PREWEANED DAIRY CALVES DURING SUMMER AND WINTER.

[1]Orellana Rivas, R. M., T. Rodrigues, J. Silveira, V. Lacerda, J. Gao, D. Ferreira de Araújo, J. Souza, T. N. Marins, P. Melendez J. K. Bernard, S. Tao. To be submitted to *Journal of Dairy Science.*

Abstract

To evaluate the effect of milk replacer (MR) feeding rate (FR) and frequency (FF) on performance, health, abomasal emptying, nutrient digestibility and glucose metabolism during the summer and winter in southeastern U.S, Holstein calves (n = 48/season) were enrolled at 7 d of age during summer (June to Aug, BW = 40.64 ± 0.65) and winter (Nov to Jan, BW = 41.86 ± 0.75). Within season, calves were randomly assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement including 2 FR (0.68 [LOW] or 0.79 kg of solid/d [HIGH] of a 26% CP and 17% fat MR), and 2 FF (2× [0700 and 1600 h] or 3× [0700, 1600 and 2200 h] daily). Calves were housed in polyethylene hutches and managed similarly throughout the trial. Milk replacer (12.5% solids) was fed to calves based on their respective treatments until d 42 when MR allowance was reduced by 50% and offered 1×/d (0700 h) for the following 7 d until weaning. Calves remained on trial until d 63. Calf starter and water were offered ad libitum. Ambient temperature and relative humidity inside and outside hutches were measured hourly. Starter and MR intake were recorded daily. Respiration rate and rectal temperature were recorded 3×/wk. Structural growth and BW were measured weekly. Plasma was collected weekly at 1400 h to analyze metabolites and insulin. Acetaminophen (50 mg/kg of BW) mixed with MR were fed to a subset of calves (0700 h, n = 10/treatment/season) on d 21. Plasma was collected at -15, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 420, and 480 min relative to feeding to analyze acetaminophen. The time for plasma acetaminophen to reach maximum (Tmax) was used to evaluate the abomasal emptying rate. A subset of calves (n = 8/TRT/season) was subjected to an intravenous glucose tolerance test (GTT) on d 28 & 29 and insulin challenge (IC) on d 29 & 59 of age at 1000 h. Plasma was collected at -15, -5, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120 min relative to glucose or insulin infusion to analyze glucose, and insulin. During summer, feeding

3× reduced preweaning respiration rate. Increasing feeding rate improved preweaning BW gain, ADG, and structural growth. There was no treatment effect on feed digestibility. Increasing FR had no effect on Tmax during the acetaminophen test, however regardless of season, increasing FF from 2× to 3× lowered Tmax. During both seasons, FF did not affect basal glucose, however feeding 2× increased basal insulin. In the summer, compared with 2×, feeding MR 3× did not affect postprandial glucose but lowered insulin, whereas in the winter, increased glucose but did not affect insulin. Following GTT in both seasons, 3× reduced insulin increment and area under the curve (AUC) compared with 2×. During IC in the summer, 3× lowered glucose AUC than 2×. Increasing FF in the summer, lowered respiration rate and rectal temperature, however no effect was detected on ADGor feed digestibility. Feeding more frequently accelerated abomasal emptying and feeding more milk replacer delayed abomasum emptying in the summer only. Increasing FF improved insulin action at the peripheral tissue level.

Introduction

Calves raised during summer have reduced growth, increased disease incidence and higher mortality rates relative to those raised in the temperate environments or thermoneutral conditions (Stull et al., 2008, Broucek et al., 2009). The reduced ADG observed in the summer can be in part attributed to heat stress. On many dairy operations, calves are often fed a fixed amount of milk or milk replacer (MR) twice daily and ad libitum starter grain, and weaned around 7-8 wks of age (USDA, 2016). When exposed to heat stress, starter intake is reduced (Chavez, 2011), resulting in lower total energy and nutrient consumption. Additionally, animals under an adverse environment experience dramatic physiological and behavioral changes to cope with distress. For example, under an environment with elevated ambient temperature (≥ 20 °C), dairy calves increase sweating and panting to dissipate heat and maintain core body temperature

(Gebremedhin et al., 1981). These physiological responses, although beneficial for survival, shift energy available for growth to maintenance. Coupled with the lower starter consumption, calves raised during summer have limited energy available for growth (Chavez, 2011).

Therefore, increasing energy intake by feeding more milk or MR should support greater growth during summer. Indeed, our previous study reported that feeding 0.65 kg DM/d of a MR containing 26% CP and 17% fat (**26:17**, as-fed basis) twice daily improved body weight gain of preweaned calves than those fed 0.55 kg of DM/d a 20:20 MR in the summer (Orellana Rivas et al., 2020). However, increasing MR (26:17) allowance above 0.65 kg of DM/d had no additional improvement on growth but was associated with increased incidence of abomasal bloating (Orellana Rivas et al., 2020). These results are inconsistent with data obtained from experiments conducted in temperate environments, where feeding large amount of milk replacer twice daily improves calf growth without reported digestive disorders (Chapman et al., 2016, Hill et al., 2016a). In temperate environment, increased milk or MR feeding rate slows abomasal emptying in dairy calves (Burgstaller et al., 2017). Although related reduced energy and nutrient utilization, the delayed abomasal emptying is considered as one mechanism for the calf to regulate the large influx of nutrients into the small intestine and has insignificant impact on growth (van den Borne et al., 2006). Heat stress slows the passage rate of digesta through the gastrointestinal tract in dairy cattle (McDowell et al., 1969). This may further delay abomasal emptying when feeding large amounts of MR during heat stress leading to increased energy loss and disturbed digestion. These may explain the lack of growth improvement and increased digestive disorders of calves fed higher amounts of MR in our previous study (Orellana Rivas et al., 2020).

Milk replacer allowance also influence calf's metabolic responses. Previous research suggest that feeding large quantities of MR increases insulin release after intravenous glucose challenge without influencing glucose clearance (Bach et al., 2013a). Similarly, we observed that increasing MR allowance during the summer increased plasma insulin concentration but did not affect glucose concentration (Orellana Rivas et al., 2020). These data indicated that feeding large quantities of MR increased the pancreatic sensitivity to release insulin and possibly resulted in reduced insulin action on peripheral tissues. Altered tissue insulin sensitivity may influence the glucose utilization for growth and have long term impacts on the body composition. Therefore, strategies need to be developed to minimize insulin resistance, improve energy utilization and growth rate while maintaining optimal health of preweaned calves raised during heat stress conditions in the southeastern United States.

Increasing FF reduces meal size and increases abomasal emptying (van den Borne et al., 2006). This theoretically reduces the energy expenditure caused by gastric hypertrophy and energy loss during abomasal fermentation, such as carbohydrates, thereby improving the efficiency of energy utilization. In veal calves, increasing MR FF from 2 to 4×/d improved retention of energy and protein (van den Borne et al., 2006, 2007). Milk replacer FF also influences glucose metabolism. In veal calves, increasing milk or MR FF from 2×/d to 4-6×/d decreased plasma glucose and insulin concentrations after a meal (Kaufhold et al., 2000, Vicari et al., 2008). This suggested well-controlled postprandial hyperglycemia. However, similar research has never been conducted in in tropical and sub-tropical environment. Such research should provide feasible recommendations that dairy producers can use to improve calf wellbeing and growth during heat stress conditions.

Therefore, our hypothesis was that calves fed large quantities of MR and raised during summer have delayed abomasal emptying, and that increasing MR FF will accelerate abomasal emptying, improve nutrient utilization, and increase glucose partition to peripheral tissues, thereby increasing calf growth. These effects would be more pronounced during heat stress conditions encountered during the summer compared with the more temperate winter common to GA. The objective of this study was to evaluate the effect of MR FRFF on performance, health, abomasum emptying, nutrient digestibility and glucose metabolism during summer and winter in southeastern U.S.

Materials and methods

Animal handling and experimental design

The experiment was conducted at the Dairy Research Center of the University of Georgia - Tifton Campus. Experimental procedures were approved by the University of Georgia Institutional Animal Care and Use Committee before beginning the study. The experimental design and procedures were performed in two seasons: summer from May to October 2018 and winter from November 2018 to April 2019. Ninety-six (n = 48/season) newborn calves were enrolled in the study. Within 6 h after birth, all calves were separated from the dam, navel dipped in 7% tinctured of iodine (Vetericyn® Super 7+, Innovacyn, Inc., Rialto, CA), and fed colostrum replacer containing at least 200 g IgG (Bovine IgG colostrum replacer, Land O'Lakes Inc., Arden Hills, MN). At 7 d of age (DOA), calves were dehorned using a caustic paste (Dehorning paste, H. W. Naylor Company, INC., Morris, N.Y.). The day of birth was considered as 1 DOA.

Starting at 2 DOA, calves were fed 0.65 kg of DM/d of a 26:17 MR (Provimi North America Inc., Brookville, OH) twice daily (0700 and 1600 h) until 7 DOA. At 8 DOA, calves were weighed and randomly assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement.

Treatments included 2 MR FR (0.65 [**LOW**] or 0.76 [**HIGH**] kg of DM/d of the 26:17 MR) and 2 MR FF (**2×** [0700 and 1600 h] or **3×**/d [0700, 1600, and 2200 h]). These resulted in 4 treatment combinations (n=12/treatment/season): **LOW2×** (summer: 5 heifers, 7 bulls; winter: 6 heifers, 6 bulls]), **LOW3×** (summer: 6 heifers, 6 bulls; winter: 5 heifers, 7 bulls), **HIGH2×** (summer: 6 heifers, 6 bulls; winter: 5 heifers, 7 bulls), and **HIGH3×** (summer: 6 heifers, 6 bulls; winter: 5 heifers, 7 bulls). The FR (0.65 and 0.76 kg of DM/d) was selected based on our previous study. Orellana Rivas et al. (2020) reported that preweaned calves fed the same 26:17 MR at a rate 0.76 kg of DM/d had similar growth performance compared with those fed 0.65 kg of DM/d during summer. And feeding MR more than 0.76 kg of DM/d with 2×/d resulted in increased incidence of abomasal bloating (Orellana Rivas et al., 2020). All calves were managed similarly and housed in individual polyethylene hutches bedded with sand in an open area without shade. Bedding was cleaned twice daily and replaced once weekly.

Feeding, intake, and growth measurements

Milk replacer was medicated with lasolocid and the main ingredients included dried whey, dried milk protein, dried whey products, and vegetable and animal fat. Textured calf starter (Godfrey's Warehouse Inc., Madison, GA) and water were offered ad libitum from 2 DOA and throughout the experiment. Water buckets were cleaned twice daily, and water refilled as needed during the day. Milk replacer was reconstituted with warm water (43 - 45 °C) to 12.5% solids and divided into equal amount per feeding according to feeding frequency. At 43 DOA, MR feeding rate was reduced by half and offered once daily (0700 h) for all calves until 49 DOA. Calves remained in the hutches until 56 DOA, and then transferred to individual stalls in a ventilated barn until the end of the experiment at 63 DOA. Calf starter intake was measured daily, and the amount of MR refused was recorded to calculate MR intake. Representative calf starter and MR samples were collected weekly and pooled monthly for chemical analysis at Cumberland Valley Analytical Services (Waynesboro, PA) (Table 4.1). Growth was assessed by measuring body weight (BW), body length, withers height, and hip height on a weekly basis (1000 h).

Environmental conditions, rectal temperature, respiration rate, fecal score, and morbidity

During the experiments, environmental temperature and relative humidity were measured every 15 min using Hobo Series Temp probes (Onset Computer Corporation, Pocasset, MA). To evaluate the environment inside the hutch, a Hobo probe was hung in the center of an empty hutch approximately 0.75 m above the ground. The ambient environment outside the hutch was assessed using another Hobo probe installed on a wood fence post adjacent to the hutches. Rectal temperature was measured using a thermometer (20-s digital thermometer, 144-920-000, ReliOn; Mabis Healthcare Inc., Waukegan, IL) and respiration rate assessed by counting flank movements for 1 min for all calves on Monday, Wednesday, and Friday each week (1430 h). Fecal score was recorded twice daily (0700 and 1600 h) based on fecal fluidity ranging from 0 (normal) to 3 (watery) (School of Veterinary Medicine, University of Wisconsin–Madison, 2011).

Disease incidence (scours, respiratory diseases, bloating, and navel, ear or cutaneous infections) were recorded for all calves. Scour was diagnosed by the appearance of loose feces as described above, reduced MR intake, and dehydration. The afternoon MR feeding of scouring calves was replaced with electrolytes (Re-Sorb; Zoetis Services LLC, Parsippany, NY) for 3 to 5 d until recovery. Calves with respiratory problems were identified by difficult breathing, coughing, fever, and the appearance of nasal discharge. When antibiotic treatments were required, recommendations from the farm veterinary were followed.

Blood sampling and metabolites analyses

Blood samples were collected from all calves at 1400 h on 7, 14, 21, 28, 35, 42, 49, 56 and 63 DOA via jugular vein into sodium-heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and immediately put on ice. Samples were centrifuged at 2,619 × g at 4 °C for 30 min to collect plasma. Plasma concentrations of glucose (Autokit glucose; Wako Chemicals USA Inc., Richmond, VA) and insulin (Bovine insulin ELISA; Mercodia AB, Uppsala, Sweden) were determined using commercially available kits; the inter and intra-assay coefficient of variation were 4.23 and 8.17%, and 5.63 and 4.17%, respectively.

Apparent total tract nutrient digestibility, fecal and feed analyses

During the pre- (14.9 ± 1.1 DOA) and postweaning (51.0 ± 1.1 DOA) periods, a subset (n = 8/treatment/season) of calves used to determine the apparent digestibility of nutrients, using chromic oxide as the external marker. Starting 5 d prior to until the end of fecal sampling, calves received 1.18 g (preweaning period) or 2.35 g DM (postweaning period) of Cr_2O_3 (Fisher Scientific, Fair Lawn, NJ) per day. The amount of Cr_2O_3 were equally divided into 2 portions that were delivered orally with gelatin capsules using a bolus gun (Torpac Inc. Fairfield, NJ) at 1000 and 2200 h each day. Eight fecal grab samples per calf were collected via rectal palpation to accommodate 3-h intervals (0000, 0300, 0600, 0900, 1200, 1500, and 1800 h) of the day by alternating sampling times over a 5 d period. Samples of fresh feed (MR and starter) and orts were collected twice daily (0700 and 1400 h) on days fecal samples were collected. Samples were placed in a forced air oven at 55 °C for 48 h to determine DM. Dry samples (starter, orts and feces) were pooled by calf and grounded to pass through a 1 mm screen for analysis of ash (method 942.05, (AOAC, 2005)), CP (method 990.03, (AOAC, 2006)), aNDFom (method 2002.4 + burning of fibrous residue at 550 °C for 2 h, (AOAC, 2002)), crude fat (method 954.02, (AOAC, 2005)), starch (method 2014.10, (AOAC, 2014)), and sugars (Dubois et al., 1956). Chemical analysis of MR samples was performed at Cumberland Valley Analytical Services (Waynesboro, PA). Fecal Cr_2O_3 concentrations were analyzed as described by Fenton and Fenton (1979) at the Agricultural & Environmental Service Lab of the University of Georgia. The ratio technique using chromic oxide as an inert marker was used to estimate apparent nutrient digestibility (DM, OM, CP, crude fat, starch, sugar, and aNDFom).

Acetaminophen test, blood sampling, and laboratory analyses

Abomasal emptying rate was determined using acetaminophen in a subset (n = 10/treatment/season) of calves at 20.1 ± 1.2 DOA (mean ± SD). On the day before the acetaminophen test, following the afternoon feeding (~1700 h) a catheter (14G×2" Radio-Opaque, Surflash™ I.V. catheter, Ref: SR*FF1451, TERUMO® Corporation, Somerset, NJ) was inserted into the jugular vein of each calf. During the morning feeding, calves were fed MR mixed with 50 mg/kg BW of acetaminophen (N-acetyl-4-aminophenol, Sigma-Aldrich, St. Louis, MO). Blood samples were collected through the catheter into vacutainer tubes containing sodium fluoride and potassium oxalate (Becton Dickinson, Franklin Lakes, NJ) at -15, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 420, and 480 min relative to the starting point of MR feeding. Samples were immediately put on ice and then centrifuged at 2,619 × g at 4 °C for 30 min to collect plasma. The catheter was flushed with sterile saline containing sodium heparin (10 IU/mL) after each sampling to prevent clotting, the first 2 mL of blood collected was discarded before each sample. Plasma concentration of acetaminophen was assessed by a colorimetric assay using a commercially available kit (Paracetamol assay kit, Cat#: K8001, Cambridge Life Science, Cambridgeshire, UK) and inter- and intra- coefficient of variance was 8.48 and 9.28%, respectively. To assess postprandial metabolic responses, glucose, and insulin concentrations were analyzed in plasma samples collected at -15, 15, 30, 60, 90, 120, 180, 240, 300, 420 min relative to feeding using the same methods described previously; the inter and intra CV were 7.42 and 3.62%, and 6.7 and 6.0% for glucose and insulin, respectively.

Metabolic test and laboratory analyses

A subset of calves (n = 8/treatment/season) was subjected to intravenous glucose tolerance test (GTT) and insulin challenge (IC) during the pre- and postweaning periods. Calves were at 26.9 ± 1.3 and 57.2 ± 1.3 DOA and at 27.9 ± 1.1 and 57.9 ± 1.1 DOA when the GTT and IC were performed, respectively. On the day of GTT, immediately after the MR feeding (~0730 h), a catheter was inserted into the jugular vein of calves and remained until the end of IC. At ~1030 h, calves received a bolus of glucose (0.3 g/kg of BW [(Monteiro et al., 2016)], dextrose 50%, wt/vol; Phoenix Scientific Inc., St. Joseph, MO) or insulin (0.1 IU/kg of BW, Novolin® R, 100 IU/mL, recombinant human insulin, Novo Nordisk Inc., Plainsboro, NJ) through the catheter inserted in the jugular vein followed by 10 mL of sterile saline containing 10 IU/mL heparin to flush the catheter and prevent clotting. The first 2 mL of blood collected was discarded before each sample. Blood samples were collected through the catheter into vacutainer tubes containing sodium fluoride and potassium oxalate (Becton Dickinson, Franklin Lakes, NJ) at -15, -5, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, and 120 min relative to the glucose infusion during GTT. And blood samples were collected at -15, -5, 0, 15, 20, 30, 40, 50, 60, 75, 90, and 120 min relative to insulin infusion during IC. Immediately after sampling, blood tubes were place and in ice then centrifuged at 2,619 × g at 4 °C for 30 min to collect plasma. Plasma concentrations of glucose and insulin were determined using commercially available kits as described previously. For samples collected in GTT, the inter- and intra-CV were 16.8 and 4.4% and, 6.8 and 4.9% for glucose and insulin, respectively, and for samples collected in IC, the inter- and intra-CV were 6.2 and 3.7%, and 7.1 and 4.5% for glucose and insulin, respectively.

Calculation and statistical analyses

A plot of plasma acetaminophen concentration-by time was generated for each calf to determine the maximum plasma concentration of acetaminophen and the time to reach the maximum concentration. The time to reach the maximum plasma concentration of acetaminophen (Tmax) is highly correlated with the abomasal emptying rate measured by nuclear scintigraphy, a gold standard for measuring gastric emptying (Marshall et al., 2005) and was used as the primary parameter to estimate abomasal emptying.

For data collected from metabolic tests, the average concentration of plasma glucose or insulin for the samples collected at -15, -5, and 0 min relative to infusion was considered as baseline. The baseline values were subtracted from the maximum or minimum concentrations to obtain the increment or decrement change in glucose or insulin when appropriate. The area under the curve (AUC) between times was calculated using the trapezoidal method, where the concentrations of glucose or insulin was calculated by subtracting the baseline value from the actual glucose or insulin concentrations. The accumulated AUC of glucose and insulin were calculated from 5-30 min, 5-60 min, and 5-120 min for GTT, and from 15-30 minutes, 15-60 min, and 15-120 min for IC. Exponential curves of glucose concentration from 5-60 min of the GTT and from 15-40 min for the IC were fitted using PROC NLIN of SAS, and the clearance rate (CR) and time to reach half of the maximal concentration ($T_{1/2}$) were calculated using the following equations: $CR\%/min = 100 \times (\ln|t_a| - \ln|t_b|)/(t_b - t_a)$; and $T_{1/2}\ min = 100 \times [\ln(2)]/CR$, were $|t_a|$ and $|t_b|$ are the concentrations at time a and b, respectively (Hayirli et al., 2001).

Data from each season were analyzed separately. Environmental temperature and relative humidity were summarized by the UNIVARIATE procedure of SAS 9.4 (SAS Institute Inc., Cary, NC), and the means ± standard deviation are reported. The MIXED procedure of SAS 9.4 was used to analyze repeated measures data, including intake (MR, starter, total DM, fat, CP, ME), ADG, fecal score, and blood metabolites and insulin. Fixed effects in the SAS models included sex, FR, FF, time, and their interactions with calf (FR × FF) as the random variable. The GLM procedure of SAS 9.4 was used to analyze the changes in BW, hip and withers heights and body length, apparent total tract nutrient digestibility within each period (pre and postweaning), and Tmax following acetaminophen infusion. The model included sex, FR, FF, and their interactions. The least square means ± standard error of the mean are presented. Incidences of diseases was analyzed using the LOGISTIC procedure of SAS 9.4, and the day of first incidence of scour was analyzed using the GLM procedure of SAS 9.4.

Plasma concentrations of glucose, and insulin relative to treatments were analyzed by PROC MIXED of SAS 9.4 as repeated measures data. The statistical model included sex, FR, FF, time, and their interactions with calf (FR × FF) as at random variable.

Data collected from metabolic tests were analyzed by periods (pre- and postweaning). The GLM procedure of SAS was used to analyze baseline, maximum, minimum, increment or decrement, CR, $T_{1/2}$, and accumulated AUC of glucose and insulin in each test. The model included sex, FR, FF and their interaction. Plasma concentrations of glucose and insulin were analyzed as repeated measures data using PROC MIXED of SAS. The statistical model included sex, FR, FF, minute relative to infusion, and their interactions with calf (FR × FF) as the random variable. The least square means ± standard error of the means are reported. Significance and tendency were declared when $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

Results

Environments, rectal temperature, respiration rate and health

Chemical analyses of MR and calf starter are detailed in Table 4.1. The CP and fat contents of MR as percentage of DM used in summer and winter averaged 27.98 and 19.01%, and 26.22 and 18.37%, respectively (Figure 4.1). During the summer, the averages of environmental temperature and relative humidity were 26.14 ± 2.24 °C and 83.85 ± 7.78% outside the hutch, and 26.98 ± 2.54 °C and 83.35 ± 7.57% inside the hutch, respectively. During the winter, environmental temperature and relative humidity averaged 12.85 ± 5.42 °C and 81.54 ± 15.18% outside the hutch, and 13.27 ± 5.56 °C and 83.00 ± 14.00% inside the hutch, respectively (Figure 4.1). Treatments had no ($P > 0.11$) impacts on respiration rate or rectal temperature during the post-weaning period. During summer, increasing FF from 2× to 3×/d reduced ($P = 0.01$) respiration rate during the preweaning period regardless of MR FR, but only lowered ($P = 0.02$) the rectal temperature of calves fed HIGH (FR × FF: $P = 0.04$). In contrast, increasing FF tended ($P = 0.06$) to reduce rectal temperature but did not affect respiration rate of preweaned calves in the winter (Table 4.2).

During summer, there was a FR × FF interaction ($P < 0.01$) for fecal score observed at 2 - 4 wk of age. This was because increasing FF reduced ($P < 0.01$) the fecal score only for calves fed LOW (Table 4.2). Treatments had no effects on the incidences of scours, pneumonia, or other causes of morbidity in both seasons ($P \geq 0.15$, data not shown).

Intakes and growth

In both seasons, FF and FR had no ($P > 0.10$) effect on intakes during the postweaning period. Consistent with the experimental design, HIGH calves consumed more MR than LOW ($P < 0.01$, Table 4.3 and 4.4). As expected, calves fed HIGH had greater ($P \leq 0.02$) intakes of CP, fat, and ME before weaning compared with LOW in both seasons. No difference was observed for FF (*P* > 0.10) on intakes during summer. However, there was a trend for and interaction of FR and FF (*P* = 0.08) for MR intake, such that increasing FF from 2× to 3×/d tended (*P* = 0.06) to increase MR intake for LOW calves but not for HIGH calves (Table 4.3). In contrast, during winter, HIGH calves fed 2×/d consumed more (*P* < 0.01) MR than 3×/d, but FF did not (*P* = 0.90) affect MR intake of LOW calves (FR × FF: *P* = 0.02; Table 4.4). Regardless of the FR, feeding 3×/d increased (*P* ≤ 0.05) the intakes of starter, DM, CP and ME before weaning relative to 2×/d during winter (Table 4.4).

During summer, FF, FR and their interaction had no (*P* > 0.10) effect on growth during the postweaning period. Relative to LOW, feeding HIGH increased (*P* ≤ 0.02) ADG and gains in BW, hip height, and body length before weaning (Table 4.5). Regardless of the FR, during summer, increasing FF from 2× to 3×/d tended (*P* = 0.10) to increase the gain of hip height from 2 to 6 wk of age. During winter, calves fed HIGH had greater (*P* ≤ 0.07) ADG and gains in BW and body length before weaning compared with those fed LOW (Table 4.5). In contrast to summer,feeding MR 3×/d in the winter tended (*P* ≤ 0.07) to increase ADG and BW gain before weaning and increased (*P* = 0.04) the gain of wither height during the postweaning period compared with 2× (Table 4.5). Compared with LOW, feeding HIGH increased (*P* ≤ 0.07) ADG/DMI, ADG/CP, and ADG/ME before weaning in both summer and winter (Tables 4.3 and 4.4). Compared with 2×/d, calves fed 3×/d tended to have lower (*P* ≤ 0.08) ADG/DMI and ADG/ME before weaning in the summer but not in the winter (Table 4.3).

Apparent digestibility test and acetaminophen pharmacokinetics

During summer, no differences were observed among treatment in apparent digestibility of DM, OM, or other nutrients during the preweaning or postwenaing periods (*P* ≥ 0.12; Table

4.8) except that calves fed HIGH tended (*P* = 0.10) to have greater fat digestibility during the postweaning period than those fed LOW. In the winter, an interaction of FF by FR (*P* = 0.06) for fat digestibility was observed for both pre- and postweaning periods. This is because the fat digestibility of calves fed HIGH but not LOW tended to be increased (*P* = 0.10) before weaning but was lowered (*P* = 0.08) after weaning when FF increased from 2× to 3×/d.

Time to reach maximum plasma acetaminophen concentration (Tmax) following acetaminophen ingestion was used to evaluate abomasal emptying. As expected, increasing MR FF from 2× to 3×/d lowered ($P \leq 0.01$) the Tmax during both summer and winter, indicating accelerated abomasum emptying (Table 4.7, Figure 4.2a, b). In the summer, calves fed HIGH tended (*P* = 0.10) to have a longer time to reach the maximum plasma acetaminophen concentration compared with LOW (Figure 4.2a). In contrast, there was no effect of FR effect (*P* = 0.56) on Tmax in the winter (Figure 4.2b).

Basal and postprandial glucose and insulin

Preweaning plasma glucose concentration was higher ($P \leq 0.04$) for calves fed HIGH compared with LOW during both summer and winter (Table 4.6). However, MR allowance did not affect ($P > 0.15$) plasma insulin concentration before weaning or circulating glucose or insulin concentrations after weaning. During the preweaning period, increasing FF from 2× to 3×/d lowered ($P \leq 0.01$) plasma insulin concentration in both seasons without ($P \geq 0.17$) affecting plasma glucose concentration (Table 4.6). After weaning, , calves previously fed 3×/d had higher plasma glucose concentration compared with those previously fed 2×/d only during winter (*P* = 0.03; Table 4.6). Milk replacer FF had no effect ($P \geq 0.56$) on plasma glucose concentrations after weaning in both seasons.

Glucose and insulin concentrations measured during the acetaminophen test. were not affected by FR during both seasons ($P \geq 0.20$; Table 4.7, Figure 4.2). However, compared with 2×, feeding 3×/d in the winter increased (P =0.02, Table 4.7, Figure 4.2d*)* glucose without affecting (P = 0.45, Table 4.7) insulin concentrations (Figure 4.2f). During the summer, FF did not affect glucose (P = 0.93, Table 4.7, Figure 4.2c), however insulin release post-feeding was lower for 3×/d compared with 2×/ d, (P =0.01, Figure 4.2f)

Metabolic tests

Before weaning, CR, $T_{1/2}$, and AUC of glucose following GTT was not different among treatments in either season except that calves fed LOW tended (P = 0.07) to have higher AUC_{30} than HIGH during winter (Table 4.9 & 4.10, Figure 4.3b). In response to intravenous glucose infusion, preweaning calves fed 3×/d had lower ($P \leq 0.05$) maximal concentration, increment, and AUC of plasma insulin compared with calves fed 2×/d in both winter and summer (Tables 4.9 & 4.10, Figure 4.3c & d). After weaning, the response to GTT differed among treatments during summer and winter. In the winter, FR, FF or their interaction had no ($P \geq 0.18$) effect on CR, $T_{1/2}$, and AUC of plasma glucose or the increment and AUC of circulating insulin following GTT of calves after weaning. In contrast, during summer, calves previously fed 3×/d tended (P = 0.10) to have faster glucose CR, greater ($P \leq 0.05$) glucose AUC, and higher ($P \leq 0.04$) increment and AUC of circulating insulin compared with those previously fed MR 2×/d (Tables 4.9 & 4.10, Figure 4.3).

In both seasons, FR, FF or their interaction had no effects on CR, $T_{1/2}$, and AUC of insulin before and after weaning during IC (Tables 4.11 & 4.12, Figure 4.5 & 4.6). Compared with those fed 2×/d, preweaning calves fed 3×/d had greater ($P \leq 0.02$) decrement and AUC of plasma glucose after IC in both summer and winter. During the postweaning period, treatments did not ($P \geq 0.10$) affect parameters related to glucose responses following IC of calves raised in the winter. However, during summer, interactions of FR and FF ($P \leq 0.04$) were observed for decrement, AUC_{60} and AUC_{120} of circulating glucose. These were because calves fed HIGH 3×/d had the greatest values compared with other treatments (Table 4.11).

Discussion

Calves reared during summer are exposed to challenging environment because of the heat stress conditions. Calves increase respiration and sweating rates when ambient temperature exceeds 20 °C (Gebremedhin et al., 1981), . During the summer, ambient temperature was consistently higher than 20 °C within a day and calves had considerably high average rectal temperatures (39.35 °C) and respiration rates (70 breaths/min). Normal rectal temperature of calves in thermal neutral condition is 38.5 °C (Collier et al., 2018). These changes confirmed that calves were experiencing heat stress. Although lower than summer, calves in the winter had the average rectal temperature (39.07 °C) higher than 38.5 °C as well. These may suggest that calves raised during the winter also experience some degree of heat stress and carried extra heat load. Indeed, during the 134 d experiment the average ambient temperature exceed 20 °C on 12 d and 48 d had maximal ambient temperature exceeding 20 °C. Therefore, during the Georgia winter, calves were not exposed to traditional cold stress. This weather pattern represents the normal climate in the southeast region of the US. Additionally, calves' body temperature exhibits a diurnal pattern with greater body temperature in the afternoon than morning (Piccione et al., 2003, Hill et al., 2016a). In the current study, the rectal temperature was measured at~1430 h which could not represent the daily average body temperature. Techniques that continuously measure body temperature within a day will provide a better evaluation of calves' thermal status.

During summer, feeding MR 3×/d reduced rectal temperature compared with 2×/d for calves fed LOW before weaning but not for those fed HIGH. Although no differences were observed in the incidence of scours, feeding LOW3× lowered fecal score compared with feeding LOW2× within the first 4 wk of age. This not only suggested that increasing FF may alleviate the severity of scour for LOW calves, but also explained the reduced body temperature due to lower feverish responses. However, it is not clear why FF did not affect the fecal score of HIGH calves. Interestingly, feeding 3×/d in the summer reduced respiration rate compared with 2×/d during the preweaning period, suggesting these calves had a lower heat load to be dissipated. During winter, increasing FF tended to lower rectal temperature without affecting fecal score or respiration rate. These data indicate that calves fed frequently had lower heat load. This may be due to reduced metabolic heat production, or increased heat dissipation or both. Indeed, van den Borne et al. (2006) reported that feeding 4×/d reduced heat production on a metabolic BW basis of veal calves compared with 2×/d regardless of intake.

As expected, increasing FF substantially accelerated abomasal emptying in both seasons, because of reduced meal size. MacPherson et al. (2016) reported that increasing FR from 0.6 to 1.2 kg of solid/d reduced abomasal emptying rate under non heat-stressed condition. In contrast, FR had no impact on abomasal emptying rate during winter. This suggested that the small increase in meal size was not a limiting factor for abomasal emptying in winter. However, increasing FR tended to delay abomasal emptying under heat stress conditions. Heat stress limits gastric motility in dairy cattle and was reported to reduce the rate of passage for lactating dairy cows and prepubertal heifers compared with those under thermal neutrality (McDowell et al., 1969, Nonaka et al., 2008). Our results suggest that severe heat stress may also delay abomasal emptying in preweaned calves when FR is increased. It is worth noting that other stressors such as calf diarrhea have also been shown to delay abomasal emptying (Kirchner et al., 2015, Hildebrandt et al., 2017).

Consistent with the experimental design, in both seasons, calves fed HIGH had higher MR intake resulting in greater CP, fat and ME intake compared with LOW during the preweaning period. In contrast to previous studies (Quigley et al., 2018, Dennis et al., 2019) that reported reduced starter intake when FR was increased, in our current experiment FR did not affect starter intake in both pre- and postweaning periods. Compared with Quigley et al. (2018) who fed 0.66 or 1.07 kg/d and Dennis et al. (2019) who fed0.66 or 0.85 kg of DM/d, calves in the current study were fed 0.65 and 0.76 kg/d of MR. The smaller difference in MR allowance may have prevented us from observing differences in starter intake. Increasing FR improved growth before weaning in both seasons, likely because of the greater intake of energy and total nutrients. Consistently, it was reported that higher FR improved preweaning ADG and skeletal growth by increasing the energy and protein intake (Brown et al., 2005, Blair, 2015). Milk replacer feeding rate did not affect growth performance after weaning. Previous researchers have reported that increasing the FR reduced digestibility of nutrients provided by grain and altered diurnal feeding pattern after weaning leading to reduced growth (Miller-Cushon et al., 2013, Quigley et al., 2018, Dennis et al., 2019). In the current study, neither starter intake nor nutrient digestibility after weaning was affected by FR.

Increasing FR improved ADG/DMI ratio during the preweaning period, consistent with previous studies (Diaz et al., 2001, Brown et al., 2005, Bartlett et al., 2006). The greater feed efficiency is a result of the larger dilution effect of maintenance cost of the calf (Diaz et al., 2001, Bartlett et al., 2006, van den Borne et al., 2006). In both seasons, calves fed HIGH had greater ADG/CP and ADG/ME but similar ADG/fat before weaning compared with those fed

LOW. Similarly, we previously reported that preweaning ADG had the lowest correlation with total fat intake compared with total DM, CP and ME intakes during summer (Orellana Rivas et al., 2020). These data indicate the importance of dietary intakes of ME and protein for improving growth of preweaning calves. However, the increased ME intake for greater ADG cannot be achieved by increasing fat content of the MR. Hill et al. (2009) reported that calves fed 0.66 kg DM/d of MR containing 27% CP with different fat concentrations (14-23%) had similar feed efficiency during both pre- and postweaning periods.

The effect of milk or MR FF on starter intake and growth of dairy calves has been inconsistently reported. An early study reported that feeding whole milk 2×/d at 450 g of solid/d increased starter intake and BW gain before but not after weaning compared with 1×/d (Schingoethe et al., 1986). In contrast, Stanley et al. (2002a) fed a MR containing 22% CP and 15% fat at 10% of initial BW at the beginning of the trial either 1× or 2×/d, and reported that FF had no effect on starter intake or growth. When compared with 2×/d, feeding 4×/d had no effect on starter intake when a MR containing 26% CP and 18% fat were fed at 2% (MR solid) of BW, but increased the starter consumption shortly before and during weaning when a 20:20 MR were fed at a rate of 1.5% of BW (Kmicikewycz et al., 2013). However, growth was not affected by FF by MR composition (Kmicikewycz et al., 2013). Sockett et al. (2011) reported that,, calves fed a MR 2×/d containing 28% CP and 20% fat (1.135 kg of solid/d) 3×/d increased the starter intake during the week of weaning when MR allowance was reduced by half and fed 1×/d. Increasing FF improved gains in body weight and hip height before weaning (Sockett et al., 2011). In contract, a recent study (MacPherson et al., 2016) reported that FF (2 vs. 4×/d) had no effects on starter intake or growth when calves were fed a 26% CP and 18% fat MR at a rate of 1.2 kg of solid/d and raised on an automated calf feeder. The reasons for the discrepancy among studies include but not limited to allowance and composition of MR, and the interval between feedings. Results from our current suggest that weather also influenced the calf's responses to FF.

During winter, increasing FF improved starter intakes before weaning, increasing DM, ME, and CP intakes. Feeding MR 3×/d increased ADG but not skeletal growth before weaning compared with 2×/d, suggesting improved adipose tissues and/or muscle growth. Consistently, in veal calves, increasing FF from 2 to 4×/d improved energy retention as fat and protein (van den Borne et al., 2006). Similar effects were not observed during summer, which may reflect the inhibitory effects of heat stress on intake and growth.

Abomasal emptying is a critical factor that regulates energy and nutrient utilization in preweaning calves. For example, increasing MR allowance increases meal size and delays abomasal emptying reducing the efficiency of energy and nutrients utilizations. This is because of the gastrointestinal hypertrophy to cope with the large meal sizes (MacPherson et al., 2016), increased abomasal fermentation of carbohydrates (Burgstaller et al., 2017), and increased amino acid oxidation because temporal plasma amino acid concentrations after feeding exceed the requirement for protein retention (van den Borne et al., 2006, 2007). In contrast, the accelerated abomasal emptying by increasing FF has been reported to lower heat production, improve energy and protein retention, and reduce amino acid oxidation (van den Borne et al., 2006,2007). With faster abomasal emptying in this current study, we expected that calves fed MR 3×/d would have improved energy and nutrient utilization compared with those fed 2×/d during both seasons. However, increased FF during winter had no impact on feed efficiency but improved body weight gain before weaning. In contrast, calves fed 3×/d during the summer had lower ADG/ME than calves fed 2×/d without influencing growth. These data potentially suggest that increasing

FF alters energy partitioning towards maintenance rather than growth before weaning. The routes of energy use may include heat dissipation that could partially explain the lower heat load as indicated by lower rectal temperature of calves with increased FF.

There was no effect of FR on apparent nutrient digestibility before weaning during summer and winter. Consistently, veal calves fed MR at 1.5 or 2.5 × metabolizable energy for maintenance had similar nutrient digestibility (van den Borne et al., 2006). In contrast, increasing the FR of a 27:18 MR from 0.66 to 1.31 kg of solid/d enhanced digestibility of DM, OM, fat, and CP at wk 3 after birth (Hill et al., 2016b). These data suggest feeding higher amount of MR had no negative effect on MR digestion. The reduced digestibility of nutrient provided by starter grains has been observed in calves offered large amount of MR (Weary et al., 2008, Hill et al., 2016a, Dennis et al., 2018, Dennis et al., 2019). This was largely attributed to delayed rumen development resulting from lower starter intake (Warner et al., 1956), (Tamate et al., 1962, Church, 1988). In the current study, increasing FR from 0.65 to 0.76 kg DM/d of a 26:17 MR did not affect starter intake or digestibility of DM, OM, CP, sugar and starch provided by calf starter in both seasons. During summer, calves previously fed HIGH tended to have greater fat digestibility after weaning compared with those fed LOW. This may suggest that increase in MR allowance improved absorption of fat provided by calf starter during summer but not winter. Interestingly, calves with FR during the winter tended to have greater digestibility of aNDFom after weaning, potentially indicating altered rumen function. However, FR did not affect starter intake before or after weaning, suggesting similar rumen development. Therefore, the mechanisms of the altered fiber digestion by increased MR allowance are still unknown. The impact of FF on apparent nutrient digestibility is seldom reported. Veal calves fed 4×/d had similar nutrient digestibility as those fed 2×/d (van den Borne et al., 2006). Similarly, FF did not influence nutrient digestibility provided by MR and starter grains in both seasons, except for fat during winter. Increasing FF increased the fat digestibility provided by MR but reduced the fat digestibility of calf starter only for calves fed HIGH during winter. The reasons for the altered fat digestibility are unknown and its impact on calf growth is also not clear.

Regardless of season, increasing FR increased basal plasma glucose concentration before weaning without affecting insulin concentration, possibly due to the higher lactose consumption by greater MR intake. In contrast, our previous study conducted in summer (Orellana Rivas et al., 2020) reported that calves fed HIGH had similar plasma glucose and higher insulin concentration compared with calves fed LOW. The discrepancy between studies may be due to the time of blood sample collection (1400 h in this current study vs. 1100 h in (Orellana Rivas et al., 2020) relative to the morning feeding (0700 h in both studies). Increasing FF from 2 to 3×/d did not affect plasma glucose concentration but decreased circulating insulin concentrations during the preweaning period in both seasons. This, coupled with a higher glucose to insulin ratio, may suggested that feeding MR more frequently reduced insulin release and enhances peripheral tissue insulin action before weaning. Either FR or FF had no impact on basal glucose and insulin concentration after weaning during summer. In contrast, in the winter, calves previously fed MR more frequently had greater plasma glucose concentrations after weaning. This may suggest increased hepatic gluconeogenesis, reduced glucose utilization or both. However, because the growth was not affected by treatments in both seasons, basal glucose concentrations do not seem to be associated with body growth after weaning in this experiment.

During the preweaning period in both seasons, calves fed MR 3×/d had similar glucose clearance but reduced insulin responses following GTT than those fed 2×/d. These data indicated that increasing FF diminished pancreatic insulin release after glucose stimulation without affecting glucose disposal. Moreover, calves fed MR more frequently had stronger glucose responses following IC. This, coupled with similar insulin clearance, suggested that increasing FF enhanced insulin mediated glucose entry into the peripheral tissues, such as muscle and adipose tissues, of preweaned calves. This increased peripheral tissue insulin action might be a negative feedback response to the reduced insulin release, and explains similar glucose disposal after GTT between calves fed MR 2 and 3×/d. In addition, seasons did not affect these responses. In contrast, Stanley et al. (2002) reported that increasing FF from 1 to 2×/d increased insulin release to intravenous glucose infusion without influencing peripheral tissue insulin sensitivity. When raised on an automated calf feeder and fed large quantities of MR (1.2 kg solids/d from a 26% CP and 18% crude fat MR), calves entitled MR access 4×/d had similar glucose clearance after GTT without influencing insulin response and tissue sensitivity compared with those entitled MR access 2×/d (MacPherson et al., 2016). The reasons for the discrepancy among studies may include but not limited to the amount of MR offered, the composition of MR and the feeding methods.

Before the morning MR feeding in both seasons, calves fed 3×/d had higher plasma concentration of glucose due to the shorter interval from last MR feeding (9 vs. 15 h, respectively) relative to calves fed 2×/d. This may also explain the higher preprandial plasma insulin concentration of calves fed 3×/d than those fed 2×/d. Similarly, MacPherson et al. (2016) reported higher preprandial plasma glucose concentration with calves' entitled access 4×/d to an automated calf feeder than those entitled 2×/d. Although numerically higher in calves entitled MR more frequently, plasma insulin concentrations before feeding was not affect by feeding frequency (MacPherson et al., 2016). It is important to note the number of animals per feeding frequency (5 vs. 20) in the experiment conducted by MacPherson et al. (2016) is lower than the current experiment.

The postprandial glucose metabolism of the milk-fed calves is also significantly affected by FF. Compared with those fed 1×/d, preweaned dairy calves fed MR 2×/d had similar postprandial plasma glucose concentration but reduced insulin concentrations (Stanley et al., 2002). Similarly, MacPherson et al. (2016) reported that preweaned dairy calves raised on an automated calf feeder and entitled with MR access 4×/d had similar postprandial plasma glucose concentration but lower insulin concentrations than those entitled with MR access 2×/d at wk 4 of age. In contrast, in large veal calves (BW ≈ 110-140 kg), feeding milk or MR more frequently than 2×/d was associated with reduced postprandial plasma glucose and insulin concentrations (Kaufhold et al., 2000, Vicari et al., 2008). The discrepancy between studies may be due to the higher MR allowance and older age (11-14 vs. 8 wk of age) of veal calves than preweaned dairy calves. Interestingly, in this current study, two distinct patterns of postprandial circulating glucose concentration were observed between calves with different MR feeding frequency in different seasons.

During summer, calves with different FF had similar postprandial plasma glucose concentration, consistent with results reported by Stanley et al. (2002) and MacPherson et al. (2016). However, calves fed MR more frequently had higher plasma glucose concentrations from 30 to 180 min after morning feeding during winter. This is particularly surprising because calves fed 3×/d received less MR, thus less lactose intake, per meal relative to calves fed 2×/d. Because no differences were observed for glucose disposal following GTT, the higher postprandial plasma glucose concentrations may suggest faster nutrient delivery to small intestine and intestinal glucose absorption because of the faster abomasal emptying rate of calves fed MR more frequently in the winter. The reasons for the distinct patterns of postprandial plasma glucose concentration between FF in different seasons are not clear but may reflect the increased glucose usage for maintenance during heat stress. During lactation, heat-stressed dairy cows had increased glucose utilization as an energy source in peripheral tissues or cells rather than mammary gland (Baumgard and Rhoads, 2012). Additionally, previous studies (Tao et al., 2012, Monteiro et al., 2016) observed that maternal heat stress during the last 45 d of gestation increased glucose disposal after intravenous glucose infusion of the preweaned calf. Similar increase in glucose utilization by pre- and postnatal calves raised during summer would occur and mask the potential differences in postprandial plasma glucose with different FF. Similar to glucose concentrations, the postprandial plasma insulin concentrations displayed district patterns in calves fed 2 vs. 3×/d in different seasons. However, the circulating insulin concentration seemed to be dependent on plasma glucose concentrations and reflected reduced insulin secretion in response to glucose stimulation of calves fed MR more frequently.

Peripheral tissue insulin sensitivity and pancreatic insulin release are critical components of glucose metabolism in dairy calves and are proposed to influence hepatic function, body growth and composition, and future performance (Gerrits et al., 2008, Van Eetvelde and Opsomer, 2017). It is imperative to consider if altered insulin sensitivity by nutrition or management in the preweaning period could persist after weaning. During summer, the greater glucose response after IC suggested that the increased peripheral tissue insulin action of calves fed MR 3×/d persisted after weaning compared with those fed 2×/d, especially for calves with greater MR allowance. Following GTT after weaning, calves previously fed MR 3×/d had greater insulin response compared with calves previously fed 2×/d. This contrasted with our observations with different FF before weaning and suggested that the pancreas of calves might develop a compensatory response after the treatment ceased. The greater insulin release to glucose stimulation and stronger insulin action on peripheral tissues explained the faster glucose disposal after GTT of the postweaned calves previously fed MR more frequently. Future research needs to examine the enhanced tissue insulin action by FF could persist beyond 8 wk of age, and how it would affect the calf's postweaning body growth, fat deposition, health and future performance. In contrast, in the winter, no difference in glucose or insulin responses were observed between calves with different FF in both metabolic tests after weaning. These suggested that the altered metabolic responses by increased FF diminished after the treatment ceased during winter. The mechanisms why altered peripheral tissue insulin sensitivity between calves with different FF only persisted after weaning in calves raised in summer is not clear but warrants further investigation.

In both seasons, FR had no impacts on glucose or insulin responses to either GTT and IC before and after weaning. In contrast, feeding large quantities of MR at 2×/d to veal calves caused hyperglycemia, hyperinsulinemia, and insulin resistance in peripheral tissues as a negative feedback mechanism of prolonged elevation of plasma insulin (Doppenberg and Palmquist, 1991, Hostettler-Allen et al., 1994, Hugi et al., 1997). In preweaned dairy calves, increasing FR increased insulin response after GTT without affecting glucose clearance, suggesting insulin resistance (Bach et al., 2013b, Yunta et al., 2015, MacPherson et al., 2016). Additionally, in this present experiment no effect of FR was observed on pre- and postprandial glucose and insulin concentrations. In large veal calves, increasing FR (19.5% CP and 20% fat) from 1.5 to 2.5 × metabolizable energy requirements for maintenance increased pre-prandial insulin but not glucose concentrations, and substantially increased plasma glucose and insulin concentrations after feeding (Vicari et al., 2008). In preweaned dairy calves, feeding a MR containing 24% CP and 18% fat at a rate of 1.2 kg of solid/d increased postprandial insulin responses but not glucose responses compared with those fed 0.6 kg of solid/d (MacPherson et al., 2019). The lack of differences in glucose metabolism between calves with different FR in the current study may be due to the smaller differences in FR used in this study. Compared with previous studies where calves with higher FR consumed 1.5-2 time more MR than control animals (Bach et al., 2013b, Yunta et al., 2015, MacPherson et al., 2016), HIGH calves only consumed 14-16% more MR than calves fed LOW. Our previous study (Orellana Rivas et al., 2020) suggested that increasing FR more than HIGH 2×/d during summer in southern GA could lead to increased incidence of abomasum bloating. This prevented us from feeding an aggressive amount of MR (> 0.76 kg of solid/d) to calves enrolled in this current study.

Conclusion

Ambient temperatures during the winter in the southeastern U.S. are variable and temperatures above 20 °C are common. Therefore, calves raised in the winter during this experiment experienced some degree of heat stress as evidenced by their elevated rectal temperature. Although there was no impact on ADG, increasing FF during summer seemed to alleviate heat stress by reducing the calves' respiration rate. Increasing FF accelerated abomasal emptying rate in both seasons and increasing FR only delayed abomasal emptying rate in the summer, confirming our hypothesis that heat stress results in delayed abomasal emptying. Milk replacer allowance had no impact on postweaning grain intake and nutrient digestibility. Increasing FF only, increased starter intake and ADG in the winter. These may reflect the inhibitory effect of heat stress on feed intake and growth in the summer. Data from the metabolic test indicate that increased FF reduced the pancreatic insulin release to glucose stimulation and enhanced insulin action on peripheral tissues before weaning in both summer and winter.

However, the increased peripheral tissue insulin action of calves fed 3×/d only persisted after weaning during summer but not winter. Future research is warrant to examine the effect of milk or FF on glucose metabolism beyond 8 wks of age and its effect on future reproductive and lactation performance.

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Table 4.1. Chemical analysis (mean ± SD) of milk replacer (MR) and calf starter

Item, % of DM unless noted	MR (n=6) Summer	MR (n=5) Winter	Calf starter (n=6) Summer	Calf starter (n=6) Winter
CP	27.98 ± 1.51	26.22 ± 0.67	17.77 ± 0.66	18.43 ± 1.33
Fat	19.01 ± 1.03	18.37 ± 1.32	4.13 ± 0.58	4.17 ± 1.38
Lactose[1]	46.18 ± 1.78	48.91 ± 0.68	-	-
ADF	-	-	10.98 ± 0.67	10.77 ± 0.21
Ash-free NDF	-	-	19.67 ± 1.26	18.09 ± 1.13
NFC	-	-	52.55 ± 1.18	51.00 ± 1.86
Ash	6.82 ± 0.48	6.42 ± 0.21	6.56 ± 0.29	6.81 ± 0.87
ME[1], Mcal/kg of DM	4.81 ± 0.06	4.76 ± 0.06	3.04 ± 0.03	3.02 ± 0.06
Calcium	0.82 ± 0.10	0.78 ± 0.04	0.88 ± 0.11	0.91 ± 0.24
Phosphorous	0.65 ± 0.05	0.61 ± 0.03	0.50 ± 0.03	0.51 ± 0.03
Magnesium	0.09 ± 0.01	0.09 ± 0.00	0.27 ± 0.01	0.26 ± 0.03
Potassium	1.41 ± 0.07	1.48 ± 0.09	1.52 ± 0.05	1.51 ± 0.11
Sodium	0.65 ± 0.05	0.58 ± 0.02	0.25 ± 0.02	0.39 ± 0.19
Iron, mg/kg of DM	85.67 ± 44.84	54.00 ± 19.33	263.33 ± 41.93	255.50 ± 35.99
Manganese, mg/kg of DM	38.67 ± 25.46	21.2 ± 10.06	112.17 ± 26.69	89.17 ± 20.66
Zinc, mg/kg of DM	16.83 ± 2.93	16.00 ± 1.41	162.17 ± 21.83	111.50 ± 22.35
Copper, mg/kg of DM	6.67 ± 1.63	8.50 ± 7.29	22.33 ± 6.62	20.67 ± 5.16

[1]Calculated according to NRC (2001).

Table 4.2. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on rectal temperature, respiration rate and fecal score during summer and winter

	Treatment[12]					*P*-value		
	LOW		HIGH		SEM			
Item	**2×**	**3×**	**2×**	**3×**		**FR**	**FF**	**FR × FF**
Summer								
Rectal temperature, °C								
Wk 2-6	39.35[a]	39.16[b]	39.25[ab]	39.29[ab]	0.05	0.79	0.18	0.04
Wk 7-8	39.60	39.56	39.47	39.60	0.08	0.56	0.62	0.31
Wk 2-8	39.42	39.28	39.32	39.38	0.05	0.96	0.43	0.06
Respiration rate, breaths/min								
Wk 2-6	70.99	62.80	73.79	63.07	3.33	0.66	0.01	0.73
Wk 7-8	70.53	69.76	68.14	73.38	4.62	0.90	0.63	0.52
Wk 2-8	72.78	67.41	73.87	67.69	3.25	0.83	0.08	0.90
Fecal score								
Wk 2-4	1.23[a]	0.90[b]	1.02[bc]	1.13[ac]	0.07	0.92	0.09	<0.01
Wk 5-8	0.13	0.17	0.10	0.19	0.03	0.90	0.12	0.52
Wk 2-8	0.60[a]	0.50[b]	0.50[b]	0.57[ab]	0.04	0.62	0.61	0.03
Winter								
Rectal temperature, °C								
Wk 2-6	39.17	39.00	39.10	39.08	0.05	0.94	0.06	0.15
Wk 7-8	39.07	38.94	39.03	39.00	0.07	0.82	0.25	0.47
Wk 2-8	39.14	38.98	39.09	39.06	0.05	0.82	0.05	0.18
Respiration rate, breath/min								
Wk 2-6	43.53	43.63	47.56	44.93	1.71	0.13	0.48	0.44
Wk 7-8	52.05	50.74	49.37	53.95	2.86	0.94	0.57	0.31
Wk 2-8	45.81	45.55	48.09	47.34	1.77	0.25	0.78	0.90
Fecal score								
Wk 2-4	1.37	1.12	1.48	1.33	0.20	0.42	0.30	0.79
Wk 5-8	0.30	0.41	0.51	0.47	0.06	0.07	0.66	0.28
Wk 2-8	0.76	0.71	0.93	0.84	0.10	0.15	0.48	0.83

[1] LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 12/treatment/season.

[2] Within a row, means without a common superscript differ (P <0.05).

Table 4.3. Milk replacer (MR) feeding rate (FR) and frequency (FF) effects on intakes and efficiencies during summer

	Treatment[1]					***P*-value**		
	LOW		**HIGH**		**SEM**			
Item	**2×**	**3×**	**2×**	**3×**		**FR**	**FF**	**FR× FF**
Milk replacer, kg/d	0.56	0.58	0.66	0.65	0.01	<0.01	0.37	0.08
Starter, kg/d								
wk 2-6	0.21	0.27	0.18	0.20	0.04	0.21	0.25	0.60
wk 7-9	1.64	1.90	1.60	1.70	0.11	0.27	0.11	0.43
wk 2-9	0.75	0.88	0.71	0.76	0.06	0.23	0.14	0.48
Dry matter, kg/d								
wk 2-6	0.82	0.90	0.89	0.91	0.04	0.24	0.22	0.47
wk 7-9	1.74	2.00	1.71	1.80	0.11	0.31	0.12	0.44
wk 2-9	1.17	1.31	1.20	1.25	0.06	0.82	0.14	0.44
CP, kg/d								
wk 2-6	0.21	0.22	0.23	0.23	0.01	0.02	0.22	0.42
wk 7-9	0.32	0.36	0.32	0.33	0.02	0.33	0.12	0.45
wk 2-9	0.25	0.28	0.26	0.27	0.05	0.76	0.14	0.42
Fat, kg/d								
wk 2-6	0.13	0.13	0.14	0.14	0.002	<0.01	0.22	0.27
wk 7-9	0.09	0.10	0.09	0.09	0.004	0.50	0.15	0.46
wk 2-9	0.11	0.12	0.12	0.12	0.01	0.01	0.16	0.34
ME[2], Mcal/d								
wk 2-6	3.57	3.83	3.97	4.02	0.12	0.02	0.21	0.41
wk 7-9	5.46	6.23	5.39	5.65	0.32	0.32	0.11	0.43
wk 2-9	4.28	4.73	4.50	4.63	0.66	0.70	0.08	0.34
ADG/DMI, g/g								
wk 2-6	0.51	0.47	0.60	0.53	0.03	0.02	0.07	0.58
wk 7-9	0.43	0.46	0.44	0.47	0.03	0.69	0.30	0.86
wk 2-9	0.48	0.46	0.54	0.50	0.02	0.03	0.26	0.56
ADG/CP, g/g								
wk 2-6	2.02	1.90	2.32	2.06	0.13	0.07	0.14	0.60
wk 7-9	2.32	2.48	2.39	2.51	0.14	0.75	0.31	0.88
wk 2-9	2.13	2.12	2.34	2.23	0.10	0.09	0.51	0.60
ADG/fat, g/g								
wk 2-6	3.45	3.36	3.79	3.47	0.22	0.33	0.36	0.61
wk 7-9	8.88	9.31	9.08	9.50	0.38	0.61	0.28	0.99
wk 2-9	5.50	5.59	5.77	5.73	0.23	0.37	0.92	0.77
ADG/ME, g/Mcal								
wk 2-6	0.12	0.11	0.14	0.12	0.01	0.04	0.08	0.58
wk 7-9	0.14	0.15	0.14	0.15	0.01	0.78	0.33	0.91
wk 2-9	0.12	0.12	0.14	0.13	0.00	0.07	0.43	0.60

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 12/treatment.
[2]ME = total metabolizable energy calculated according to NRC (2001).

Table 4.4. Milk replacer (MR) feeding rate (FR) and frequency (FF) effects on intakes and efficiencies during winter.

	Treatment[1]					***P*-value**		
	LOW		**HIGH**		**SEM**			
Item	**2×**	**3×**	**2×**	**3×**		**FR**	**FF**	**FR× FF**
Milk replacer, kg/d	0.56	0.56	0.67	0.63	0.01	<0.01	0.01	0.02
Starter, kg/d								
wk 2-6	0.18	0.29	0.15	0.23	0.03	0.16	<0.01	0.58
wk 7-9	1.56	1.79	1.68	1.71	0.08	0.78	0.14	0.23
wk 2-9	0.70	0.85	0.72	0.79	0.05	0.66	0.03	0.34
Dry matter, kg/d								
wk 2-6	0.78	0.89	0.85	0.91	0.03	0.14	0.02	0.40
wk 7-9	1.67	1.85	1.79	1.81	0.08	0.62	0.22	0.33
wk 2-9	1.11	1.24	1.20	1.25	0.05	0.34	0.07	0.39
CP, kg/d								
wk 2-6	0.19	0.21	0.21	0.22	0.01	0.01	0.03	0.34
wk 7-9	0.31	0.35	0.34	0.34	0.02	0.59	0.14	0.23
wk 2-9	0.24	0.26	0.26	0.27	0.04	0.17	0.05	0.27
Fat, kg/d								
wk 2-6	0.12	0.12	0.14	0.13	0.002	<0.01	0.51	0.20
wk 7-9	0.08	0.09	0.09	0.09	0.003	0.27	0.14	0.23
wk 2-9	0.10	0.11	0.12	0.12	0.01	<0.01	0.19	0.19
ME[2], Mcal/d								
wk 2-6	3.39	3.72	3.80	3.92	0.11	0.01	0.05	0.35
wk 7-9	5.14	5.83	5.60	5.67	0.25	0.57	0.14	0.23
wk 2-9	4.05	4.50	4.43	4.56	0.16	0.18	0.08	0.35
ADG/DMI, g/g								
wk 2-6	0.39	0.41	0.49	0.50	0.04	0.03	0.72	0.86
wk 7-9	0.45	0.47	0.48	0.45	0.02	0.79	0.82	0.26
wk 2-9	0.41	0.43	0.48	0.48	0.03	0.07	0.81	0.67
ADG/CP, g/g								
wk 2-6	1.62	1.77	1.98	2.07	0.17	0.06	0.50	0.85
wk 7-9	2.34	2.44	2.50	2.36	0.08	0.66	0.80	0.15
wk 2-9	1.89	2.02	2.18	2.18	0.13	0.10	0.63	0.62
ADG/fat, g/g								
wk 2-6	2.80	3.25	3.19	3.50	0.31	0.31	0.23	0.84
wk 7-9	8.88	9.27	9.54	9.20	0.29	0.32	0.94	0.22
wk 2-9	5.08	5.49	5.56	5.63	0.27	0.26	0.37	0.52
ADG/ME, g/Mcal								
wk 2-6	0.09	0.10	0.11	0.12	0.01	0.07	0.45	0.85
wk 7-9	0.14	0.15	0.15	0.14	0.01	0.73	0.87	0.29
wk 2-9	0.11	0.12	0.13	0.13	0.01	0.06	0.54	0.53

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 12/treatment.

[2] ME = total metabolizable energy calculated according to NRC (2001).

Table 4.5. Milk replacer feeding rate (FR) and frequency (FF) effects on growth performance during summer and winter.

	Treatment[1]				SEM	*P*-value		
	LOW		HIGH					
Item	**2×**	**3×**	**2×**	**3×**		**FR**	**FF**	**FR× FF**
Summer								
ADG, kg/d								
wk 2-6	0.48	0.48	0.58	0.55	0.03	0.01	0.77	0.69
wk 6-9	0.79	0.90	0.78	0.85	0.06	0.59	0.15	0.65
wk 2-9	0.60	0.64	0.65	0.66	0.04	0.28	0.46	0.63
BW gain, kg								
wk 2-6	16.74	16.76	19.81	18.75	1.00	0.02	0.62	0.61
wk 6-9	16.51	18.92	16.48	17.80	1.22	0.64	0.13	0.66
wk 2-9	33.26	35.68	36.29	36.55	2.09	0.36	0.52	0.61
Wither height gain, cm								
wk 2-6	6.15	6.25	6.65	7.06	0.47	0.17	0.59	0.74
wk 6-9	3.74	4.33	3.94	3.79	0.33	0.60	0.50	0.27
wk 2-9	9.90	10.58	10.58	10.85	0.54	0.38	0.38	0.70
Hip height gain, cm								
wk 2-6	5.44	6.10	6.73	7.29	0.36	<0.01	0.10	0.89
wk 6-9	4.57	4.13	4.17	4.31	0.39	0.78	0.70	0.46
wk 2-9	10.01	10.23	10.90	11.60	0.59	0.06	0.44	0.68
Body length gain, cm								
wk 2-6	5.88	5.21	6.54	7.65	0.62	0.02	0.73	0.16
wk 6-9	5.09	4.79	4.67	5.31	0.54	0.93	0.75	0.39
wk 2-9	10.30	10.67	12.31	11.85	0.81	0.05	0.96	0.61
Winter								
ADG, kg/d								
wk 2-6	0.39	0.46	0.47	0.52	0.03	0.04	0.06	0.75
wk 6-9	0.73	0.85	0.84	0.83	0.05	0.34	0.27	0.16
wk 2-9	0.52	0.61	0.61	0.63	0.03	0.07	0.06	0.34
BW gain, kg								
wk 2-6	13.44	16.07	16.69	18.07	1.09	0.02	0.07	0.57
wk 6-9	15.21	17.95	17.66	17.53	0.92	0.28	0.16	0.13
wk 2-9	28.65	34.04	34.87	35.63	1.70	0.03	0.08	0.18
Wither height gain, cm								
wk 2-6	5.35	5.78	6.18	6.07	0.49	0.27	0.75	0.59
wk 6-9	3.23	4.21	3.60	4.15	0.35	0.66	0.04	0.54
wk 2-9	8.58	9.99	9.91	10.22	0.51	0.13	0.09	0.28
Hip height gain, cm								
wk 2-6	5.25	5.95	6.43	6.35	0.52	0.13	0.55	0.45
wk 6-9	3.65	3.60	4.29	4.10	0.35	0.11	0.74	0.83
wk 2-9	8.90	9.57	10.95	10.46	0.52	0.01	0.86	0.27
Body length gain, cm								
wk 2-6	4.48	6.00	6.44	6.38	0.62	0.07	0.24	0.21
wk 6-9	5.23	4.87	5.37	5.18	0.53	0.66	0.60	0.88
wk 2-9	9.71	10.88	12.01	11.57	0.79	0.07	0.65	0.31

[1]LOW = 0.65 kg DM/d of a milk replacer containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a milk replacer containing 26% CP and 17% fat; 2× = Feeding the milk replacer twice a day (0700 and 1600 h); 3× = Feeding the milk replacer three times a day (0700, 1600, and 2200 h); n = 12/treatment/season.

Table 4.6. Milk replacer (MR) feeding rate (FR) and frequency (FF) effects on basal plasma glucose and insulin concentrations during summer and winter.

	Treatment[1]					***P*-value**		
	LOW		**HIGH**		**SEM**			
Item	**2×**	**3×**	**2×**	**3×**		**FR**	**FF**	**FR× FF**
Summer								
Glucose, mg/dL								
wk 1-6	86.9	83.1	93.3	89.6	2.7	0.02	0.17	0.98
wk 6-9	71.1	76.1	72.1	74.1	2.6	0.86	0.18	0.57
wk 1-9	81.7	80.7	86.2	84.4	2.5	0.11	0.59	0.86
Insulin, µg/L								
wk 1-6	0.26	0.17	0.30	0.14	0.03	0.78	<0.01	0.22
wk 6-9	0.31	0.29	0.23	0.28	0.04	0.16	0.59	0.30
wk 1-9	0.28	0.20	0.27	0.18	0.02	0.42	<0.01	0.61
Glucose/Insulin								
wk 1-6	26	501	307	622	51	0.51	<0.01	0.24
wk 6-9	225	250	280	252	34	0.40	0.99	0.44
wk 1-9	288	398	308	446	35	0.36	<0.01	0.81
Winter								
Glucose, mg/dL								
wk 1-6	98.4	95.9	101.4	102.2	2.19	0.04	0.70	0.46
wk 6-9	76.0	82.1	75.4	80.4	2.41	0.62	0.03	0.81
wk 1-9	91.0	91.3	92.6	94.9	2.0	0.20	0.52	0.64
Insulin, µg/L								
wk 1-6	0.30	0.21	0.35	0.21	0.04	0.68	0.01	0.64
wk 6-9	0.30	0.26	0.27	0.35	0.05	0.56	0.66	0.20
wk 1-9	0.30	0.22	0.32	0.25	0.03	0.49	0.02	0.89
Glucose/Insulin								
wk 1-6	327	461	294	491	65	0.90	0.01	0.61
wk 6-9	234	318	292	233	36	0.75	0.77	0.06
wk 1-9	311	307	294	383	35	0.55	0.01	0.99

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 12/treatment/season.

Table 4.7. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on time to reach the maximal plasma acetaminophen concentration (Tmax), and glucose and insulin concentrations after the morning milk replacer feeding of calves at 21 days of age during summer and winter

	Treatment[1]					***P*-value**		
	LOW		**HIGH**		**SEM**			
Item	**2×**	**3×**	**2×**	**3×**		**FR**	**FF**	**FR× FF**
Summer								
Tmax, min	221.3	149.0	243.8	195.0	20.4	0.10	0.01	0.57
Glucose, mg/dL	85.7	84.8	86.1	87.5	2.8	0.60	0.93	0.69
Insulin, µg/L	0.73	0.42	0.79	0.42	0.13	0.89	0.01	0.84
Winter								
Tmax, min	258.0	192.0	246.0	183.0	17.9	0.56	<0.01	0.93
Glucose, mg/dL	86.2	95.9	90.8	98.4	3.6	0.32	0.02	0.77
Insulin, µg/L	0.47	0.51	0.71	0.51	0.09	0.20	0.45	0.22

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 10/treatment/season.

Table 4.8. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on apparent digestibility of nutrients during pre- (14.9 – 19.9 d of age, SD = 1.1 d of age) and postweaning (51.0 – 56.0 d of age, SD = 1.1 d of age) periods in the summer and winter

	Preweaning								**Postweaning**							
	Treatments[1,2,3]					***P*-value**			**Treatments**					***P*-value**		
	LOW		**HIGH**			**FR**	**FF**	**FR×FF**	**LOW**		**HIGH**			**FR**	**FF**	**FR× FF**
%	**2×**	**3×**	**2×**	**3×**	**SEM**				**2×**	**3×**	**2×**	**3×**	**SEM**			
Summer																
DM	85.3	87.8	88.3	87.9	2.2	0.49	0.64	0.51	71.6	76.5	73.3	74.2	2.6	0.91	0.28	0.43
OM	76.2	74.2	71.0	71.7	4.0	0.34	0.88	0.74	75.3	79.0	75.9	75.8	2.3	0.58	0.43	0.41
CP	88.9	93.2	92.6	90.4	2.3	0.85	0.65	0.17	71.3	72.2	68.4	66.9	2.7	0.14	0.89	0.65
Fat	88.9	93.2	92.6	90.4	2.3	0.85	0.65	0.17	67.6	60.5	70.8	73.0	4.6	0.10	0.60	0.32
aNDFm	-	-	-	-	-	-	-	-	26.1	45.6	34.5	30.0	7.5	0.64	0.33	0.12
Sugars	-	-	-	-	-	-	-	-	97.7	97.7	98.3	97.8	0.5	0.41	0.63	0.61
Starch	-	-	-	-	-	-	-	-	98.0	98.3	97.4	98.2	0.6	0.61	0.34	0.69
Winter																
DM	85.2	81.5	83.9	83.9	2.6	0.84	0.47	0.48	73.7	73.7	80.8	75.3	2.8	0.13	0.33	0.33
OM	89.9	89.1	89.7	91.0	1.9	0.65	0.91	0.60	75.9	76.6	82.7	76.9	2.7	0.20	0.36	0.24
CP	77.9	82.5	81.4	81.3	3.4	0.74	0.52	0.50	77.2	74.1	81.2	75.2	3.3	0.44	0.18	0.67
Fat	94.9[ab]	91.8[ab]	91.1[b]	96.1[a]	2.0	0.90	0.64	0.06	80.7[ab]	85.9[ab]	88.1[b]	77.4[a]	4.1	0.90	0.50	0.06
aNDFm	-	-	-	-	-	-	-	-	29.2	28.3	49.2	36.8	7.9	0.08	0.41	0.47
Sugars	-	-	-	-	-	-	-	-	96.0	95.3	97.4	95.2	1.2	0.55	0.24	0.54
Starch	-	-	-	-	-	-	-	-	97.5	98.2	98.4	97.7	0.4	0.66	0.90	0.09

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 8/treatment/season.

[2]Preweaning apparent digestibility was calculated based on MR intake only.

[3] Within a row, means without a common superscript differ (P <0.05).

Table 4.9. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to a glucose tolerance test of calves during the pre- (26.9 ± 1.1 d of age) and postweaning (57.2 ± 1.3 d of age) periods in the summer

	Preweaning Treatments[1]					***P*-value**			**Postweaning Treatments**					***P*-value**		
	LOW		**HIGH**						**LOW**		**HIGH**					
Item[2]	**2×**	**3×**	**2×**	**3×**	**SEM**	**FR**	**FF**	**FR× FF**	**2×**	**3×**	**2×**	**3×**	**SEM**	**FR**	**FF**	**FR× FF**
Glucose, mg/dL																
Baseline	83.7	87.2	87.5	86.5	3.9	0.69	0.75	0.57	73.2	78.4	66.6	74.7	3.8	0.19	0.10	0.70
Maximum	157.3	170.7	169.5	167.9	6.2	0.45	0.36	0.24	177.6	181.2	171.9	174.9	4.4	0.18	0.46	0.94
Increment	73.7	83.5	82.1	81.4	4.7	0.50	0.34	0.27	104.4	102.8	105.3	100.1	3.9	0.83	0.40	0.65
CR, %/min	1.43	1.48	1.38	1.44	0.14	0.77	0.70	0.98	1.32	1.49	1.28	1.61	0.15	0.80	0.10	0.61
$T_{1/2}$, min	49.4	49.1	51.4	49.6	4.5	0.79	0.82	0.87	53.5	47.5	55.5	45.5	5.0	1.00	0.12	0.69
AUC, mg×min/dL																
30 min	1337	1507	1452	1525	103	0.52	0.25	0.64	1893	1832	2048	1768	103	0.66	0.11	0.29
60 min	1417	1631	1646	1732	231	0.48	0.53	0.78	2662	2380	3016	2182	270	0.77	0.05	0.31
120 min	1242	861	1532	1252	418	0.42	0.44	0.90	2575	2103	3418	1941	394	0.39	0.02	0.21
Insulin, µg/L																
Baseline	0.78	0.34	1.01	0.42	0.14	0.28	<0.01	0.95	0.17	0.23	0.10	0.15	0.03	0.02	0.10	0.91
Maximum	8.66	6.43	10.06	7.30	0.98	0.25	0.02	0.79	1.96	3.17	1.38	2.30	0.43	0.10	0.02	0.94
Increment	7.78	5.98	9.01	6.76	0.89	0.26	0.03	0.80	1.81	2.94	1.27	2.15	0.41	0.11	0.02	0.92
AUC, mg×min/dL																
30 min	153.1	112.6	185.6	129.3	19.6	0.22	0.02	0.69	37.4	57.4	26.6	45.4	8.6	0.19	0.03	0.94
60 min	177.4	128.9	230.7	159.9	24.3	0.09	0.02	0.65	50.6	73.1	38.3	56.7	9.0	0.12	0.03	0.82
120 min	161.4	113.8	195.5	147.1	22.9	0.15	0.05	0.99	51.2	73.5	42.5	57.4	8.5	0.15	0.04	0.66

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MRthree times a day (0700, 1600, and 2200 h); n = 8/treatment.

[2]Baseline = average glucose and insulin concentrations at −15, −5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment =concentration difference between maximum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; $T_{1/2}$ = time to reach half maximal glucose concentration; AUC = area under the curve.

Table 4. 10: Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to a glucose tolerance test of calves during the pre- (27.3 ± 1 d of age) and postweaning (57.1 ± 1 d of age) periods in the winter

	Preweaning Treatments[1,3]					***P*-value**			**Postweaning Treatments**					***P*-value**		
	LOW		**HIGH**						**LOW**		**HIGH**					
Item[2]	**2×**	**3×**	**2×**	**3×**	**SEM**	**FR**	**FF**	**FR× FF**	**2×**	**3×**	**2×**	**3×**	**SEM**	**FR**	**FF**	**FR× FF**
Glucose, mg/dL																
Baseline	82.8	100.9	94.1	99.0	3.9	0.24	0.01	0.10	75.3	81.0	75.5	81.5	3.3	0.91	0.09	0.97
Maximum	177.0	184.1	179.3	182.0	7.9	0.99	0.54	0.78	181.8	182.1	181.1	191.3	6.5	0.52	0.42	0.45
Increment	94.2	83.2	85.2	83.0	7.2	0.53	0.37	0.55	106.6	101.2	105.6	109.8	6.1	0.53	0.92	0.44
CR, %/min	2.2	1.7	2.1	2.0	0.3	0.64	0.27	0.35	2.0[a]	1.7[ab]	1.4[b]	1.8[ab]	0.2	0.16	0.68	0.06
$T_{1/2}$, min	32.94	48.73	34.97	35.99	5.99	0.45	0.19	0.26	37.1	42.7	54.3	43.3	5.2	0.10	0.60	0.12
AUC, mg×min/dL																
30 min	1402	1451	1181	1165	132	0.07	0.90	0.81	1804	1739	1945	1795	123	0.43	0.39	0.73
60 min	1099	1379	764	661	317	0.11	0.78	0.55	2022	2033	2692	2136	302	0.21	0.38	0.36
120 min	812	80	-263	-160	498	0.20	0.53	0.41	1836	2050	2788	1782	447	0.45	0.38	0.18
Insulin, µg/L																
Baseline	0.85	0.54	1.33	0.65	0.27	0.34	0.08	0.69	0.17	0.19	0.15	0.19	0.04	0.73	0.46	0.83
Maximum	10.48	6.76	12.91	8.22	1.78	0.29	0.02	0.97	6.12	5.33	3.86	4.81	1.01	0.18	0.94	0.39
Increment	9.73	7.03	12.20	8.56	1.51	0.20	0.04	0.76	5.93	5.09	3.69	4.59	1.00	0.18	0.98	0.39
AUC, mg×min/dL																
30 min	200.7	130.5	236.2	160.4	29.7	0.28	0.02	0.93	101.3	89.2	63.7	82.4	17.8	0.22	0.85	0.39
60 min	236.4	127.2	265.5	174.4	31.3	0.24	<0.01	0.77	118.8	106.4	87.0	95.4	20.4	0.30	0.92	0.62
120 min	208.1	154.2	254.0	127.0	41.0	0.82	0.04	0.38	118.3	105.4	89.2	91.7	20.4	0.31	0.80	0.71

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 8/treatment.

[2]Baseline = average glucose and insulin concentrations at −15, −5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment =concentration difference between maximum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; $T_{1/2}$ = time to reach half maximal glucose concentration; AUC = area under the curve.

[3] Within a row, means without a common superscript differ (P <0.05).

Table 4. 11: Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to an insulin challenge test of calves during the pre- (27.9 ± 1.1 d of age) and postweaning (57.9 ± 1.1 d of age) periods in the summer

	Preweaning [1] Treatments[2,3]					**P-value**			**Postweaning Treatments**					**P-value**		
	LOW		**HIGH**		**SEM**	**FR**	**FF**	**FR× FF**	**LOW**		**HIGH**		**SEM**	**FR**	**FF**	**FR× FF**
Item[3]	**2×**	**3×**	**2×**	**3×**					**2×**	**3×**	**2×**	**3×**				
Insulin, µg/L																
Baseline	0.68[a]	0.45[ac]	1.05[b]	0.31[c]	0.10	0.27	<0.01	0.02	0.17	0.23	0.10	0.15	0.03	0.02	0.10	0.91
Maximum	8.66	6.43	10.06	7.30	0.98	0.25	0.02	0.79	1.96	3.17	1.38	2.30	0.43	0.10	0.02	0.94
Increment	7.78	5.98	9.01	6.76	0.89	0.26	0.03	0.80	1.81	2.94	1.27	2.15	0.41	0.11	0.02	0.92
CR, %/min	7.02	9.41	7.76	6.80	1.01	0.36	0.49	0.11	6.32[a]	15.57[b]	11.51[ab]	9.19[ab]	2.11	0.78	0.11	0.01
$T_{1/2}$, min	10.39	8.34	8.97	10.74	1.01	0.63	0.89	0.07	9.95[a]	5.15[b]	6.24[ab]	8.18[ab]	1.39	0.99	0.31	0.02
AUC, µg×min/L																
30 min	187	141	161	187	34	0.77	0.76	0.29	148	196	163	217	37	0.63	0.18	0.93
60 min	215	158	180	212	38	0.80	0.75	0.25	184	228	179	260	43	0.75	0.16	0.67
120 min	188	171	157	189	40	0.87	0.85	0.54	190	228	162	267	48	0.91	0.15	0.50
Glucose, mg/dL																
Baseline	74.4	80.2	73.6	90.6	4.0	0.23	0.01	0.16	66.1	67.4	65.3	75.1	2.4	0.16	0.03	0.08
Minimum	36.4	26.1	40.3	36.1	3.6	0.06	0.06	0.40	24.5	26.6	23.5	25.7	1.6	0.56	0.20	0.99
Decrement	-38.1	-54.1	-33.3	-54.5	3.9	0.57	<0.01	0.51	-41.7[a]	-40.8[a]	-41.8[a]	-49.5[b]	1.7	0.02	0.06	0.02
AUC, mg×min/dL																
30 min	-527	-797	-423	-803	83	0.56	<0.01	0.51	-593	-654	-597	-738	48	0.36	0.04	0.41
60 min	-1458	-2158	-1129	-2227	190	0.50	<0.01	0.30	-1700[a]	-1692[a]	-1686[a]	-2019[b]	79	0.06	0.05	0.04
120 min	-1985	-3698	-1140	-3650	381	0.25	<0.01	0.30	-2861[ab]	-2419[a]	-2736[ab]	-2996[b]	168	0.19	0.59	0.05

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding MR three times a day (0700, 1600, and 2200 h); n = 8/treatment.

[2]Baseline = average glucose and insulin concentrations at −15, −5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment/decrement =concentration difference between maximum/minimum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; $T_{1/2}$ = time to reach half maximal glucose concentration; AUC = area under the curve.

[3] Within a row, means without a common superscript differ (*P* <0.05).

Table 4.12. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to an insulin challenge test of calves during the pre- (28.3 ± 1 d of age) and postweaning (58.1 ± 1 d of age) periods in the winter

	Preweaning Treatments[1,3]					***P*-value**			**Postweaning Treatments**					***P*-value**		
	LOW		**HIGH**						**LOW**		**HIGH**					
Item[2]	**2×**	**3×**	**2×**	**3×**	**SEM**	**FR**	**FF**	**FR× FF**	**2×**	**3×**	**2×**	**3×**	**SEM**	**FR**	**FF**	**FR× FF**
Insulin, µg/L																
Baseline	0.85	0.54	1.33	0.65	0.27	0.34	0.08	0.69	0.17	0.19	0.15	0.19	0.04	0.73	0.46	0.83
Maximum	10.48	6.76	12.91	8.22	1.78	0.29	0.02	0.97	6.12	5.33	3.86	4.81	1.01	0.18	0.94	0.39
Increment	9.57	6.18	11.21	7.30	1.52	0.37	0.02	0.98	5.93	5.09	3.69	4.59	1.00	0.18	0.98	0.39
CR, %/min	7.58	6.78	6.82	6.65	0.75	0.56	0.53	0.68	7.89	8.18	8.04	7.50	0.71	0.72	0.86	0.56
$T_{1/2}$, min	9.31	11.02	10.37	10.03	1.16	0.98	0.56	0.39	9.04	8.65	8.92	9.63	0.91	0.65	0.87	0.55
AUC, µg×min/L																
30 min	213	268	194	195	29	0.12	0.35	0.36	153	160	156	143	21	0.75	0.90	0.63
60 min	232	342	226	219	37	0.09	0.18	0.13	184	190	189	181	24	0.93	0.97	0.78
120 min	200	328	211	184	45	0.15	0.27	0.10	185	196	203	190	25	0.80	0.96	0.64
Glucose, mg/dL																
Baseline	73.4[a]	92.5[b]	85.7[b]	87.1[b]	3.9	0.39	0.01	0.03	71.0	75.5	76.3	79.5	2.8	0.11	0.18	0.82
Minimum	39.4	38.1	42.1	35.5	3.2	0.99	0.23	0.41	26.2	31.8	28.7	28.7	2.1	0.89	0.19	0.20
Decrement	-34.0	-54.4	-43.6	-51.6	4.9	0.49	0.01	0.22	-44.8	-43.7	-47.6	-50.7	2.7	0.08	0.71	0.44
AUC, mg×min/dL																
30 min	-510	-782	-658	-733	115	0.67	0.14	0.40	-696	-665	-695	-750	58	0.47	0.84	0.46
60 min	-1289	-2136	-1747	-2069	253	0.45	0.03	0.31	-1865	-1801	-1956	-1990	120	0.25	0.90	0.69
120 min	-1442	-3631	-2282	-2962	491	0.86	0.01	0.14	-2911	-2554	-3053	-2984	190	0.15	0.27	0.46

[1]LOW = 0.65 kg DM/d of a MR containing 26% CP and 17% fat; HIGH = 0.76 kg DM/d of a MR containing 26% CP and 17% fat; 2× = Feeding the MR twice a day (0700 and 1600 h); 3× = Feeding the MR three times a day (0700, 1600, and 2200 h); n = 8/treatment.

[2]Baseline = average glucose and insulin concentrations at −15, −5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment/decrement =concentration difference between maximum/minimum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; $T_{1/2}$ = time to reach half maximal glucose concentration; AUC = area under the curve.

[3] Within a row, means without a common superscript differ (*P* <0.05).

Figure 4. 1: Ambient temperature and relative humidity inside and outside the hutches during summer and winter.

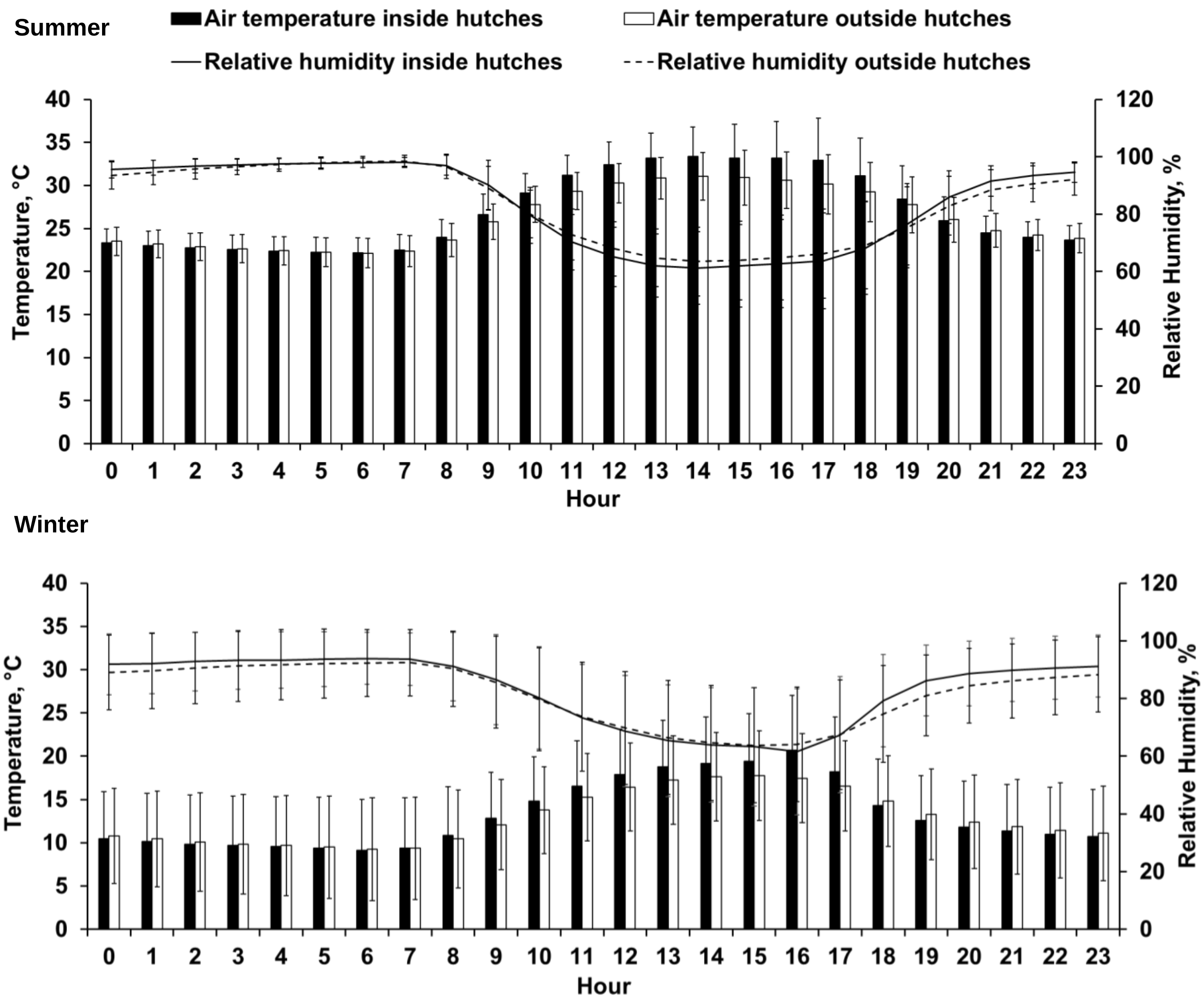


Figure 4. 2: Milk replacer feeding frequency (twice [2×, n = 20] vs. three [3×, n = 20] per d) effect on plasma acetaminophen, glucose and insulin concentrations during an acetaminophen test performed to calves (20.1 ± 1.3 d of age) during summer (a, c, e) and winter (b, d, f). Errors bars represent SEM. During summer for acetaminophen concentrations, effect of treatment (P = 0.92, minute (*P* < 0.01) and treatment by minute interaction (*P* < 0.01). During summer for the glucose concentration, effect of treatment (*P* = 0.93, minute (*P* < 0.01) and treatment by minute interaction (*P* < 0.01). During summer for the insulin concentration, effect of treatment (*P* = 0.01), minute (*P* < 0.01) and treatment by minute interaction (*P* <0.01). During winter for acetaminophen concentrations, effect of treatment (*P* = 0.08), minute (*P* < 0.01) and treatment by minute interaction (*P* < 0.01). During winter for the glucose concentration, effect of treatment (*P* = 0.02), minute (*P* < 0.01) and treatment by minute interaction (*P* = 0.03). During winter for the insulin concentration, effect of treatment (P = 0.45), minute (*P* < 0.01) and treatment by minute interaction (*P* < 0.01). ***P* ≤ 0.01, **P* ≤ 0.05, †*P* ≤ 0.10.

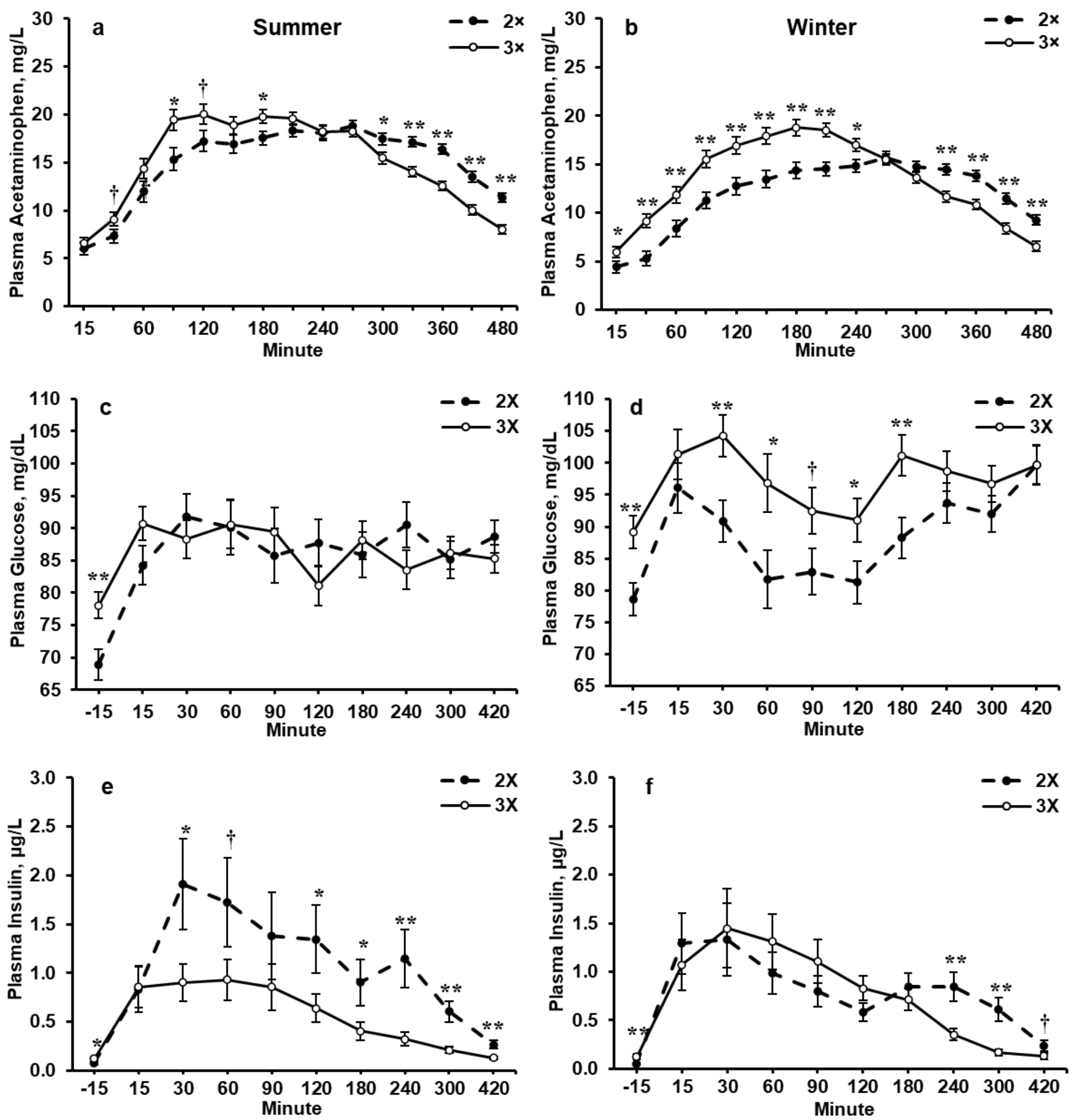


Figure 4. 3: Milk replacer feeding frequency (twice [2×, n = 16] vs. three [3×, n = 16] per d) effect on plasma glucose and insulin concentrations during a glucose tolerance test performed to calves 26.9 ± 1.1 d of age during summer (a, c) and winter (b, d).Errors bars represent SEM. During summer for the glucose concentration, effect of treatment (P = 0.82, minute (P < 0.01) and treatment by minute interaction (P = 0.24). During summer for the insulin concentration, effect of treatment (P < 0.01), minute (P < 0.01) and treatment by minute interaction (P <0.01). During winter for the glucose concentration, effect of treatment (P = 0.05), minute (P < 0.01) and treatment by minute interaction (P = 0.40). During winter for the insulin concentration, effect of treatment (P = 0.01), minute (P < 0.01) and treatment by minute interaction (P = 0.10). **P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.10.

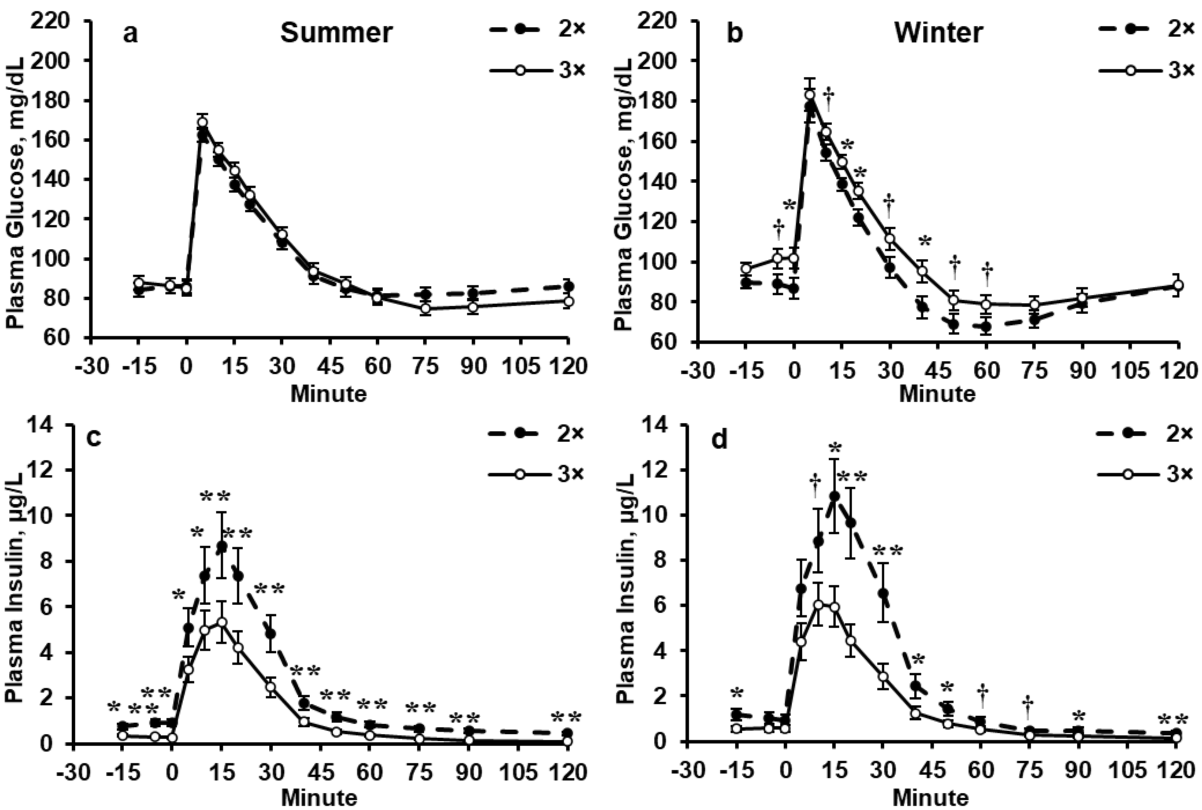


Figure 4. 4: Milk replacer feeding frequency (twice [2×, n = 16] vs. three [3×, n = 16] per d) effect on plasma glucose and insulin concentrations during a glucose tolerance test performed to calves 57.2 ± 1.3 d of age during summer (a, c) and winter (b, d). Errors bars represent SEM. During summer for the glucose concentration, effect of treatment (P = 0.91, minute (P < 0.01) and treatment by minute interaction (P = 0.10). During summer for the insulin concentration, effect of treatment (P = 0.04), minute (P < 0.01) and treatment by minute interaction (P =0.19). During winter for the glucose concentration, effect of treatment (P = 0.52), minute (P < 0.01) and treatment by minute interaction (P = 0.31). During winter for the insulin concentration, effect of treatment (P = 0.73), minute (P < 0.01) and treatment by minute interaction (P = 0.15). **P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.10.

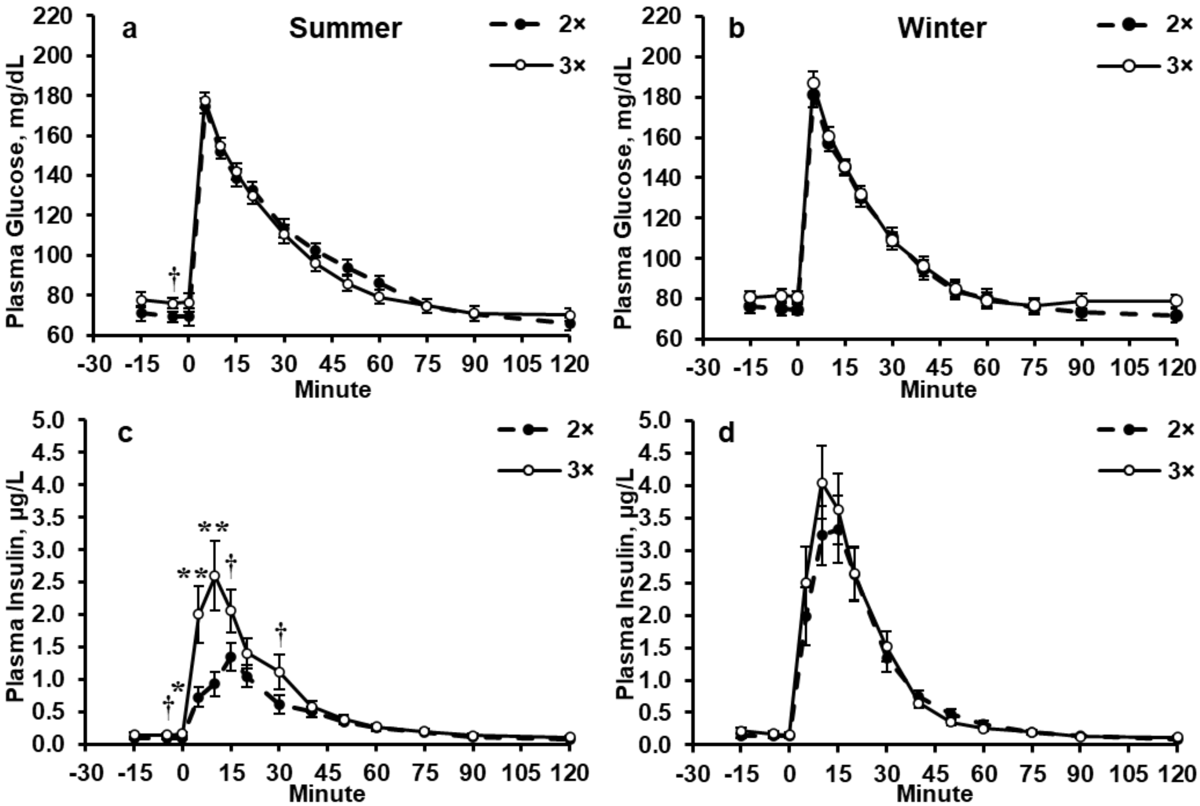


Figure 4. 5: Milk replacer feeding frequency (twice [2×, n = 16] vs. three [3×, n = 16] per d) effect on plasma glucose and insulin concentrations during an insulin challenge performed to calves 27.9 ± 1.1 d of age during summer (a, c) and winter (b, d). Errors bars represent SEM. During summer for the glucose concentration, effect of treatment (P = 0.46, minute (P < 0.01) and treatment by minute interaction (P = 0.01). During summer for the insulin concentration, effect of treatment (P < 0.01), minute (P < 0.01) and treatment by minute interaction (P = 0.01). During winter for the glucose concentration, effect of treatment (P = 0.73), minute (P < 0.01) and treatment by minute interaction (P = 0.02). During winter for the insulin concentration, effect of treatment (P = 0.23), minute (P < 0.01) and treatment by minute interaction (P = 0.17). **P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.10.

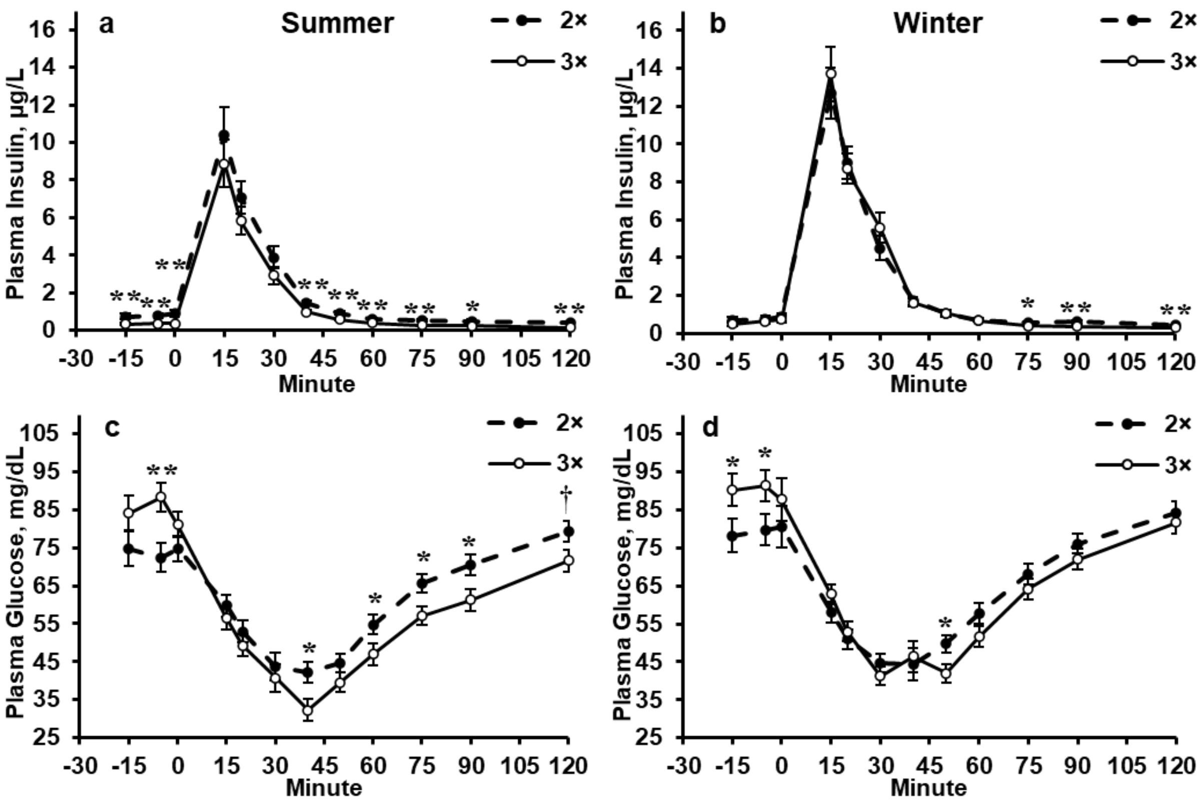
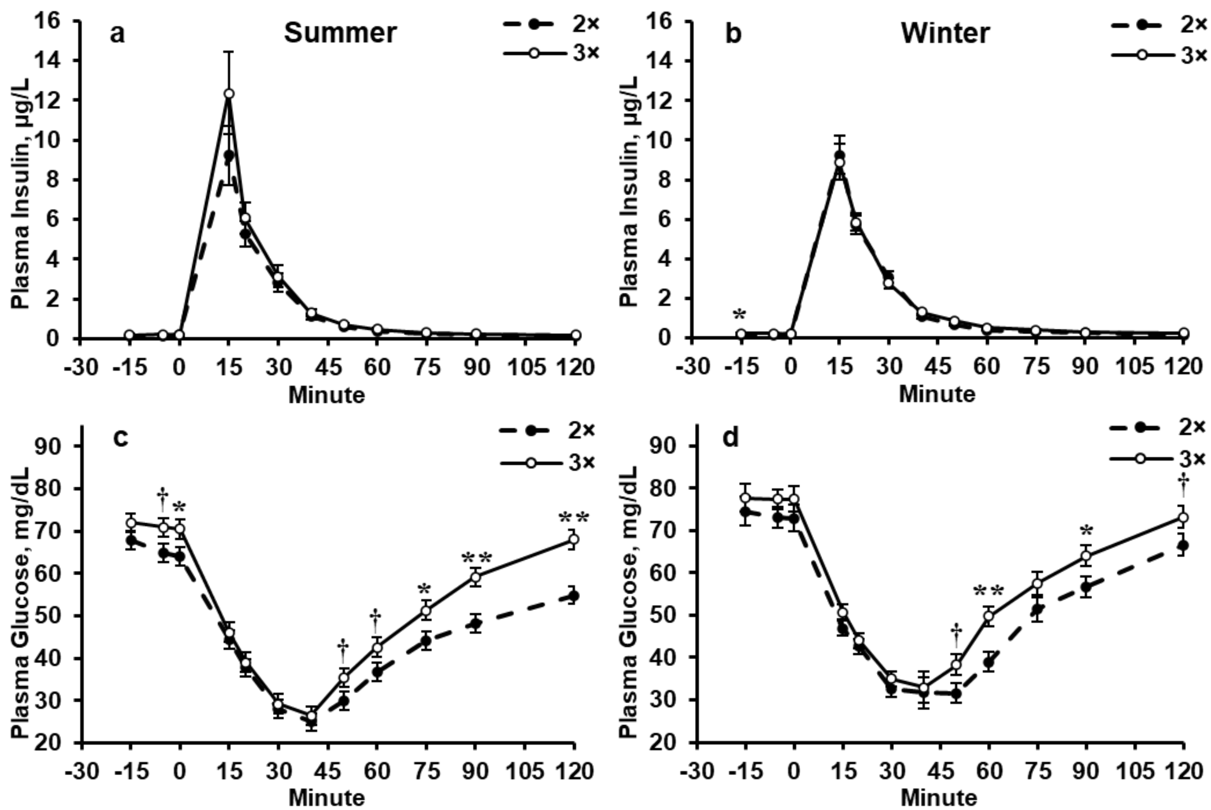


Figure 4. 6: Milk replacer feeding frequency (twice [2×, n = 16] vs. three [3×, n = 16] per d) effect on plasma glucose and insulin concentrations during an insulin challenge performed to calves 57.9 ± 1.1 d of age during summer (a, c) and winter (b, d). Errors bars represent SEM. During summer for the glucose concentration, effect of treatment (P = 0.06, minute (P < 0.01) and treatment by minute interaction (P = 0.23). During summer for the insulin concentration, effect of treatment (P = 0.16), minute (P < 0.01) and treatment by minute interaction (P = 0.98). During winter for the glucose concentration, effect of treatment (P = 0.06), minute (P < 0.01) and treatment by minute interaction (P = 0.23). During winter for the insulin concentration, effect of treatment (P = 0.11), minute (P < 0.01) and treatment by minute interaction (P = 0.48). **P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.10.



CHAPTER 5

CONCLUSIONS

Heat stress is detrimental for dairy cattle and it is critical to find strategies to minimize its impact and improve growth, development, and welfare of the animals. Calves raised under heat stress condition in tropical and subtropical environments have poor growth, impaired passive immunity and lifelong inferior performance compared with calves raised in temperate environments. Utilizing heat abatement have been reported to improve calf wellbeing, immunity, and preweaning ADG. However, nutritional and management practices to improve overall calf development are rare. In the first study, we hypothesized that increasing FR during summer would enhance performance by increasing energy intake. This would compensate for the energy loss due to the increased maintenance energy cost to reduce the negative effects of heat stress. Increasing FR from 0.55 kg of DM/d of at conventional 20:20 MR to 0.66 kg of DM/d of a 26:17 MR offered twice daily improved ADG. However, increasing MR feeding rate from 0.66 r to 0.77 kg of DM/d of the 26:17 MR had no further impact on ADG. Unexpected, feeding a more aggressive amount of MR (0.88 kg of DM/d of a 26:17 MR) caused increased gastrointestinal disorders such as abomasum bloating and diarrhea. These data suggest delayed abomasum emptying caused by larger meal size during heat stress. Furthermore, we provided preliminary evidence that increasing FR may result in insulin resistance at the peripheral tissue of preweaned calves.

The results from the first study suggested that additional approaches need to be employed to increase abomasal emptying when large quantity of MR is fed to preweaned calves during summer. Therefore, in the second study, we hypothesized that increasing FF from 2× to 3×/d will accelerate abomasal emptying, enhance insulin sensitivity and improve growth of preweaned dairy calves that are fed larger amounts of MR. These beneficial effects are more pronounced under heat stress conditions during the summer compared to the more temperate conditions during the winter. In this study, we evaluated the impact of MR feeding rate (0.66 vs. 0.77 kg of DM/d of a 26:17 MR) and frequency (2 vs. 3×/d) during summer and winter on abomasal emptying, nutrient digestibility, metabolism and growth. Increasing FF in the summer reduced heat load carried by calves because of the lower respiration rate during the preweaning period. However, no impact of FF on ADG was observed. In the winter, increasing FF slightly lowered rectal temperature, but did no effect respiration rate was observed. Regardless of seasons, increasing FF accelerated abomasal emptying due to reduced meal size. Although FR had no effect on abomasal emptying rate in the winter, calves fed more MR had delayed abomasal emptying in the summer that could be attributed to heat stress. Feeding rate or FF did not affect starter intake or digestibility of DM, CP, and fat during the pre- and postweaning periods in both seasons. Increasing FF improved insulin sensitivity at the peripheral tissue during the preweaning period, however this had no impact on ADG.